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ECOLOGIA DE *LAEONEREIS ACUTA* COMO BIOINDICADOR EM ESTUÁRIOS

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"*Ecologia de Laeonereis acuta* como bioindicador em estuários
"

Por

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RESUMO

Os poliquetas são táxons numericamente dominantes nos sistemas estuarinos, atuando como importantes elos da produção secundária alóctones e autóctones no fluxo energético rio-estuário-mar. A grande variedade e a alta densidade populacional desses organismos constituem uma fonte energética muito variada para outras espécies em redes alimentares marinhas. A baixa mobilidade e a estreita relação com o meio ambiente dão a este grupo de grandes consumidores estuarinos o status de indicadores de qualidade ambiental. No entanto, apesar de terem desenvolvido muitas habilidades adaptativas a esses ambientes, as mudanças causadas pela urbanização levaram ao desaparecimento de espécies mais sensíveis e ao domínio das mais tolerantes. A espécie *Laeonereis acuta* está distribuída por toda a costa do Atlântico Sul, sendo dominante em muitos estuários, representando assim importante fonte de energia para outros níveis tróficos. Estudos iniciais indicaram que *L. acuta* tem um comportamento oportunista devido ao seu ciclo de vida relativamente curto e estratégias de recolonização rápida de sedimentos. Outros estudos destacaram a espécie como pertencente a uma assembléia tolerante em estuários urbanizados. Mais recentemente, a potencialidade de *L. acuta* como bioindicador tolerante a poluição estimulou o mapeamento de suas alterações histológicas e morfológicas, bem como respostas bioquímicas, acumulação e biotransformação após exposição a contaminantes. Neste estudo testamos as respostas a diferentes poluentes, em diferentes níveis de complexidade biológica de *L. acuta*, por meio de aborgaens bioquímicas e ecológicas. Nossos resultados mostram que esta espécie é bom indicador da qualidade ambiental, podendo ser utilizada como bioindicador de qualidade em estuários.

Palavras-chave: Estuários. Bioindicador. Complexidade biológica. Urbanização. Produção secundária. *Laeonereis acuta.*

ABSTRACT

Polychaetes are numerically dominant taxa in estuarine systems, acting as important links of secondary allochthonous and autochthonous production in the river-estuary-sea energy flow. The great variety and high population density of all organisms constitute a diverse energy source for other species in marine food webs. Low mobility and a close relationship with the involvement of a group of large estuarine consumers or the status of environmental quality indicators. However, despite having developed many adaptive abilities to these environments, as changes caused by urbanization have led to the disappearance of more sensitive species and by the domain of the most tolerant ones. The species is distributed all along the coast of the South Atlantic, being dominant in many estuaries, thus representing na important source of energy for other trophic levels. Initial studies have indicated that *L. acuta* has a timely behaviour due to its short life cycle and rapid sediment recolonization strategies and is consider a opportunist speces. Other studies have highlighted a species as belonging to a tolerant mount in urbanized estuaries. More recently, a potentiality of *L. acuta* as a pollution tolerant bioindicator has stimulated the mapping of its histological and morphological alterations, as well as biochemical responses, accumulation and biotransmission for exposure to contaminants. This study tested as responses in different levels of biological complexity of *L. acuta*, through biochemical approachs and population dynamics. Our results show that this is a good indicator of environmental quality and can be used as a quality bioindicator in estuaries.
Keywords: Estuaries. Bioindicator. Biological complexity.
Urbanization. Secondary production. *Laeonereis acuta*.

SUMÁRIO

INTRODUÇÃO GERAL

Historicamente as atividades humanas tem sido catalisadoras des grandes transformações ambientais, com significativas alterações de natureza física ou química no solo, na atmosfera e nos oceanos. A maior parte da população humana está concentrada próxima à regiões costeiras e uma proporção significativa das maiores cidades do mundo está ligada, direta ou indiretamente, ao meio marinho (Cloern et al., 2016). Entre os ambientes costeiros, estuários são considerados os mais produtivos, com alta relevância ecológica, especialmente por constituírem ecótonos estabelecendo ligações biogeoquímicas entre o mar e o continente (Jennerjahn e Mitchell, 2013). Podem ser definidos como corpos de águas costeiros semi-fechados com uma ligação livre para mar aberto, onde a água salgada do oceano é diluída na água doce drenada do continente (Pritchard, 1967). Assim, são ecossistemas únicos, moldados por processos sazonais e espaciais. Estes ambientes desempenham, um papel vital para a biodiversidade costeira, para a saúde humana e para a civilização.

Porém, estes importantes ecossistemas têm sido exauridos pela colonização, sobreexploração dos recursos e a liberação de substâncias tóxicas ao longo dos séculos, provocando a perda da biodiversidade e da resiliência, gerando assim um cenário de colapso (Lotze et al., 2006). Regiões estuarinas em todo o mundo tem sido ao mesmo tempo fornecedoras de alimentos como moluscos e peixes, assim como também o local utilizado como área de destino de esgotos tem sido mais evidente (Winterbourn et al., 2016). Evidencia-se assim o paradoxal papel da água como um recurso vital e ao mesmo tempo um veículo para a eleiminação de poluentes.

As pressões decorrentes do uso e liberação intensiva de produtos tóxicos pela indústria agrícola, farmacêutica, petrolífera (Dixon et al., 2002; Tarouco et al., 2017) e aqueles gerados pela urbanização, exercem grande pressão sobre estes ecossistemas, (Lotze et al., 2006; Halpern et al., 2008; Merrifield et al., 2011), criando um cenário que se contrapõe a incipientes ações de fiscalização e mitigação de impactos. Como conseqüência, tem se modificado a estrutura e a função dos estuários, com expressiva queda da biodiversidade e dos bens e serviços (Halpern et al., 2008). Entre as modificações antropogênicas, a eutrofização e a liberação de poluentes alteram parâmetros físicos e químicos, aumentando a quantidade e complexidade de substâncias na água e no sedimento (Birch, 2000), transformando estuários em regiões impróprias à vida de muitas espécies, ocasionando consequências ecológicas, econômicas e sociais (Cloern et al., 2016).

No entanto, a deterioração do estado de equilíbrio do ambiente pode ter sérios efeitos sobre as associações biológicas. Nesse sentido, os estuários têm grande relevância por se tratarem de locais de abrigo, reprodução e habitat permanente de inúmeras espécies, inclusive muitas utilizadas diretamente na alimentação humana. Assim, a exposição destes organismos a contaminantes genotóxicos representa não somente um risco para a saúde humana, através da cadeia alimentar (Weber e Haig, 1997; Paiva e Silva, 1998; Martınez-Curci et al., 2015), mas também um risco ecológico especialmente pela perda de diversidade biológica e das funções do ambiente.

A utilização de bioindicadores como ferramenta para a avaliação dos impactos antropogênicos em diversas escalas espaço-temporais em ambientes costeiros têm sido amplamente difundida.

Muitos bioindicadores podem responder a impactos em diferentes níveis de organização biológica quando expostos de maneira crônica ou aguda à contaminantes. (Monserrat et al., 2007; Lopes et al., 2014).

Entre os representantes da fauna estuarina estão os poliquetas, organismos bentônicos que vivem associados ao sedimento. Promovem a bioturbação e a reciclagem de nutrientes, remobilizando e aerando o substrato, sendo tambem importante fonte de alimento para animais como caranguejos, peixes e aves. Os poliquetas são bons candidatos a bioindicadores por estarem em estreito contato com meio, possuírem ciclo de vida curto e fazerem parte da complexa rede trófica estuarina, que eventualmente inclui o próprio o homem.

A espécie *Laeonereis acuta* é encontrada em ambientes estuarinos de toda costa do atlântico Sul Americano (de 2ºS (Brasil) a 42ºS (Argentina) (Orensanz e Gianuca, 1974), apresenta grandes densidades em ambientes alterados pela ação atropogência, especialmente em estuários localizados próximos a regiões urbanizadas ou industrializadas (Pagliosa e Barbosa, 2006; Omena et al., 2012; Weis et al., 2017). Tem sido utilizada como organismo teste em ensaios toxicológicos com metais pesados e agroquímicos (Monserrat et al., 2007; Marques et al., 2013; Cordeiro et al., 2014; Tarouco et al., 2017). Outros estudos ainda evidenciaram respostas a contaminantes em tecidos e órgãos do poliqueta, assim como a capacidade antioxidante proporcionada por bactérias que utilizam o muco desta espécie como substrato (Cordeiro et al., 2014). Estudos anteriores sobre a dinâmica populacional desta espécie (Omena e Amaral, 2000, Martin e Bastida, 2006) evidenciaram que *L. acuta* constitui um abundante recurso alimentar para muitos crustáceos, peixes e aves ao longo do ano.

Do ponto de vista metodológico, o estabelececimento de relações entre a bioquímica e a ecologia ainda é um desafio. A avaliação de impactos em diferentes níveis de complexidade biológica, contemplando desde níveis moleculares até populacionais, é uma abordagem que envolve não somente ensaios em laboratório, mas também grandes esforços de campo, que requerem delineamentos robustos e que contemplem mais de uma escala local e temporal. Nesse sentido, a construção do conhecimento sobre modificações sutis, ou quando ainda se pode agir proativamente, até aquelas já estabelecidas em populações ou comunidades, depende necessariamente de observações repetidas ao longo do tempo. Portanto, o conhecimento científico e a tomada de decisão por gestores não devem estar segregadas em um momento em que se discute a conservação da biodiversidade e dos recursos naturais.

As abordagens que incluem métodos moleculares para detectar danos no material genético são procedimentos já testados e consolidados em mamíferos (Fenech, 2000), porém não se constata ainda o mesmo avanço com invertebrados (Dixon 2002). Testes para detectar clastogenicidade em poliquetas são raros, tendo em vista a dificuldade para obtenção de células mitóticas, sendo mais comuns o uso de marcadores moleculares através de enzimas, especialmente aqueles decorrentes do estresse oxidativo. A utilidade de abordagem na qual se possa quantificar danos diretamente no material genético se deve especialmente pela alta sensibilidade e rapidez na obtenção das respostas e o baixo custo do metódo. Outro aspecto importante a ser considerado está no fato de que poliquetas, assim como outros invertebrados expressam tipos qualitativamente semelhantes de danos aos encontrados em organismos superiores (Dixon, 2002; Jha, 2004).

A história de vida dos organismo pode ser formatada por inúmeros fatores, mas, sem sombra de dúvida, o tamanho corporal configura-se como um dos principais fatores. Esta é uma media alométrica importante na ecologia e tem sido relacionada tanto a características ambientais quanto fisiológicas (Peters, 1983). Embora seja uma tarefa difícil ligar respostas celulares e bioquímicas ao indivíduo, população ou comunidade, a análise comparativa tem sido uma abordagem constante para inferir a seleção natural (Smolders, 2004). Neste contexto, são esperadas diferentes respostas quanto a aspectos reprodutivos, alocação de energia, taxa de renovação somática (P/B) entre organismos da mesma espécie com diferentes tamanho, uma vez que alterações na composição corporal são expressas como alterações no orçamento energético. Portanto, o tamanho e a razão P / B são formas indiretas de medir não somente as taxas metabólicas, mas também a quantidade de energia disponível para manutenção, crescimento e reprodução (Brey, 2012).

Outro nível de abordagem inclui estimativa dos parâmetros populacionais, e a produção secundária de determinada espécie. Estes estudos fornecem informações sobre a estrutura das comunidades, fluxo de energia e também podem subsidiar ações de manejo e conservação. A estrutura das comunidades é um aspecto diretamente relacioanado às taxas de recrutamento, mortalidade, no entanto, assim como em muitos organismos, fatores extrínsecos como quantidade de alimento, propriedades do sedimento, temperatura e atividades antrópicas também podem exercer fortes influências sobre tais parâmetros, no caso dos poliquetas. Além disso, fatores intrínsecos como a capacidade adaptativa de muitos organismos em ocupar de maneira efetiva nichos vagos por outras espécies. Entre estes fatores, estão presentes habilidades de tolerar ambientes mais poluídos ou modificados pela ação antropogênica, e a forma de obtenção e transformação do alimento entre outras (Durou et al., 2007; MacDonald et al., 2012; Kelly et al., 2016).

Dessa forma, o estudo de um organismo como bioindicador deve propor-se à avaliação efetiva das respostas e o grau de complexidade dos procedimentos metodológicos para obtê-las, contemplando os diferentes níveis de complexidade biológica. Oferecendo dessa forma, oportunidade de escolha, não apenas do ponto de vista metodológico, mas também do custo e da brevidade de respostas a gestores e outros pesquisadores. Assim, cabe ressaltar o papel da espécie *L. acuta*, como bioindicadora, a partir do qual podemos obter respostas em diferentes níveis de complexidade biológica. Desta forma esta espécie pode ser utilizada como bioindicador em programas de monitoramento da qualidade ambiental de estuários.

Nesta tese, foram investigados aspectos da ecologia do poliqueta *Laeonereis acuta* (Nereididae), bem como respostas desta espécie em ambientes com diferentes níveis de perturbação antropogênica e enriquecimento orgânico. Procurando avaliar a qualidade das respostas por meio de estudos de campo e laboratório, incluindo desde níveis subcelulares até populacionais

Assim, a tese foi estruturada em três capítulos redigidos no formato de manuscritos, publicados, subemetido e em preparação para submissão. O primeiro capítulo avaliou a efeitos da urbanização em diferentes níveis de organização biológica do poliqueta *Laeonereis acuta*, por meio de experimentos laboratoriais e de campo, em estuários da região sul do Brasil. Nesse capitulo foram avalidos os danos ao material genético a partir da frequência de micronúcleos; do tamanho corporal e da biomassa individual, e a relação produção-biomassa no nível populacional. O segundo capítulo, abordou aspectos do uso dos recursos e alocação energética pelo poliqueta para o crescimento somático ou para a reprodução. Foram realizadas abordagens de dinâmica populacional, entre eles, a frequência de fêmeas férteis, taxas de mortalidade, constante de crescimento entre outras. O terceiro capítulo abordou a contaminação e os efeitos de esteróis em estuários urbanizados e não-urbanizados. Os efeitos foram medidos através dos parâmetros da dinâmica populacional da espécie.

CAPÍTULO 1

URBANIZATION EFFECTS ON DIFFERENT BIOLOGICAL ORGANIZATION LEVELS OF AN ESTUARINE POLYCHAETE TOLERANT TO POLLUTION

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2.1 ABSTRACT

Estuarine species exposed to diffuse contaminants might trigger either positive or negative feedbacks in many biological scales. Their life history traits performing at different biological organization levels could propose an organism as a useful indicator of environmental pollution, mainly addressed as sensitive or tolerant species. To track the effects of contaminants from the molecular to the population level of the polychaete

Laeonereis acuta we utilize a framework of biomarkers. For this purpose we assessed the *L.acuta* frequency of micronuclei at the molecular level, the body size and biomass at individual level, and the production-to-biomass ratio at population level in five urbanized and five non-urbanized estuaries in southern Brazil. *L. acuta* had significantly varying positive and negative feedbacks between urbanized and non-urbanized estuaries at multiple biological scales. These generalized effects in all biological organization scales indicate a pollution impact on the polychaete. The main responses accounted for individuals becoming lengthy and weighty, but with molecular damage. The *L. acuta* allocation of energy to body enlargement in polluted environments, and a consequent reduced population turnover, contradicts the expected from an opportunist species. The damages in DNA and the internal strategies of individuals, as antioxidant defense mechanisms, could favor resistance of the population and tolerance to pollutants. All of these characteristics induce bioaccumulation and could cause bottom-up pollution transfer compromising the estuarine food web. These results, ascertain that *L. acuta* could be considered as a tolerant species, instead of an opportunistic, and as a useful indicator of environmental pollution in estuaries.
Keywords: Bioindicator. Biomarker. Body size. Estuarine pollution. Micronuclei. Turnover.

2.2 INTRODUCTION

Coastal urbanization has increased the discharge of diffuse toxic substances into water bodies responsible for acute and chronic degradation of the estuarine biota. In advanced situations, this process causes elimination of sensitive species and dominance of the most tolerant or opportunistic individuals (Klerks and Weis,1986; Bickham et al., 2000; D'Alessandro et al., 2015). Along their evolutionary history, tolerant species might have selected life traits with adaptive values (fitness) that are nowadays coupled with or benefited from contaminants (Kawecki and Ebert,2004). Notwithstanding, species exposed to contaminants usually present negative responses in genetic, physiological, morphological and behavioral levels (Mersch et al., 1996; Fleeger et al., 2003; Schiedek et al., 2006; Catalano et al., 2012). The description of biological mechanisms under such evolutionary and ecological scales has been a current challenge to elucidate how species function as tolerants to environmental pollution.

Evaluation of the effects of contaminants usually involves an outlined bulk of biomarkers (Monserrat et al., 2007). To integrally comprehend those effects, the biomarkers need to be processed in many biological scales, as species may experience stress at different levels of biological organization throughout the medley from molecular to the community level. Among the implications to the environmental management of investigating many biological organization levels, the assessment of changes in lower biological levels can be used to avoid further effects on higher levels, when they have not yet happened. However, the uses and responses of biomarkers in molecular or subcellular levels in invertebrates are still poorly known and fragmented (Dixon et al., 2002). Conversely, the expression of both qualitative and quantitative damage to DNA is very similar between invertebrates and higher complexity organisms (Dixon et al., 2002; Hagger et al., 2002; Jha, 2004). These similarities could be used to drive endeavors in researches with marine invertebrates.

Polychaetes numerically dominate the estuarine communities living in the water-sediment interface, have a short lifespan and are less motile than other benthic macroinvertebrates, and thus responsive to changes in their surrounding environment. All of these characteristics confer the generic status of good indicators of environmental health (Durou et al., 2007; Botter-Carvalho et al., 2011). The Nereididae polychaete *Laeonereis acuta* is widely spreadin South American Atlantic estuaries and sheltered areas, from2°S (Brazil) to 42°S (Argentina) (Orensanz and Gianuca, 1974; Pamplin et al., 2007). Early studies have indicated *L. acuta* to have an opportunistic behavior because of their relatively short life cycle and rapid sediment recolonization strategies (Netto and Lana, 1994; Omena and Amaral, 2000). Further studies highlighted the species as pertaining to a tolerant assemblage in urbanized estuaries (Pagliosa and Barbosa, 2006). This was suggested after their density and biomass were found to be related to the general state of the environment, both in water and sediments, then simply to modifications in the sediment. More recently, the potentiality of *L.acuta* as a tolerant bioindicator of pollution has stimulated the mapping of their histological and morphological alterations, as well as biochemical responses, accumulation, and biotransformation after exposure to contaminants (Geracitano et al., 2002; Ferreira-Cravo et al., 2008; Ventura-Lima et al., 2011). However, an integrated biomarkers assessment at different levels of biological organization of *L. acuta* under urbanization effects is still lacking.

The aim of this study was to assess the effects of diffuse pollution caused by urbanization in estuaries at different biological organization levels of the polychaete *L. acuta*. For this purpose we used the biomarkers frequency of micronuclei at subcellular level, body size and biomass at the individual level, and production-to-biomass ratio at the population level. This framework was applied in urbanized and non-urbanized estuaries. Thus, we expected *L.acuta* biomarkers to indicate the environmental health diferences between estuaries types which would be detected in all levels of biological organization. It is hypothesized that *L. acuta* from urbanized estuaries will exhibit higher frequencies of micronuclei in cells, lower individual body sizes and biomass, as well as higher production-to-biomass ratio in populations when compared to those from non-urbanized estuaries.

2.3 Material and Methods

The study was conducted from August to September in 2014at ten estuaries located between the coordinates 25°5´S–48°3´W and 27°5´S–48°4W (Fig. 1). The coastal region is typically composed of bights founded by quaternary sediments turning over marine, tidal, and river-lake plains crossed by estuaries. The climate is humid subtropical with rainfall well distributed throughoutthe year. The annual mean temperature is 20◦C with seasonal differences ranging from 17 to 22◦C. The tidal regime is microtidal with average amplitude of 0.83 m for spring tides and 0.15 m for neap tides (Cruz, 1998). The estuarine basins in south Brazil are generally well preserved areas (highest concentrations recorded:1 µM of P, 52 µM of N, 27 mg/kg of Cu, 28 mg/kg of Pb, 105 mg/kg of Zn) interspersed with urbanized areas (highest concentrations recorded: 12 µM of P, 217 µM of N, 46 mg/kg of Cu, 56 mg/kg of Pb, 144 mg/kg of Zn) (Pagliosa, 2005; Pagliosa et al., 2006a,b, 2016; Rovai et al., 2013). Then, we performed a visual interpretation of aerial images complemented by field surveys, and used existente data of water and sediment quality to select. In this sense, we have determined two types of estuaries for our study in relation to the degree of anthropogenic environmental disturbance: (i) urbanized estuaries, whenever available, indicated by the water and sediment data, and when a great extent of the estuarine basins was composed of residential, commercial or industrial areas with clear signs of disturbed landscape; and (ii) non-urbanized estuaries, whenever available, indicated by the water and sediment data, and when the estuarine basin presents just a small or absence of signs of anthropic interventions and visually the physiognomy of the landscape is preserved. The study estuaries were spaced from 05 km to 200 km and belonged to a different watershed, avoiding confound effect of urbanization.

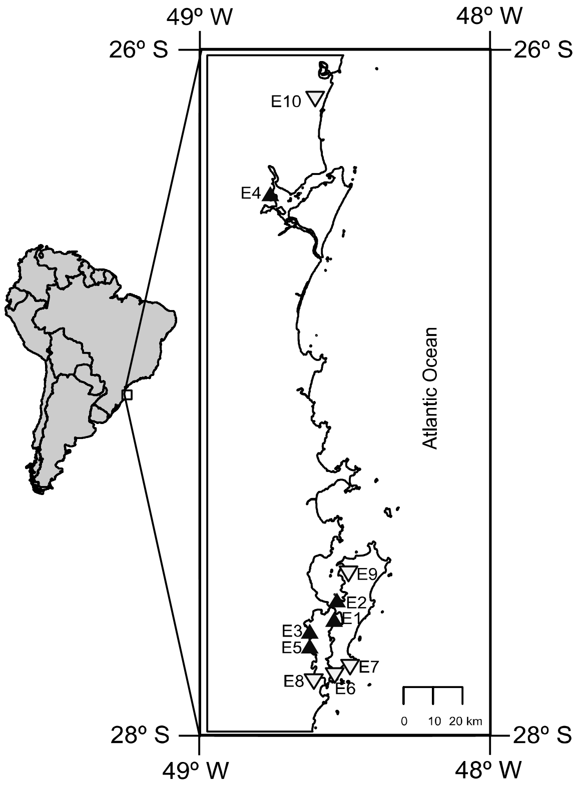


Fig. 1. Map indicating the 10 estuaries in South Brazil. E1-Costeira do Pirajubaé, E2-Itacorubi River, E3-Maruim River, E4-Saguaçu Bay, E5-Aririu River, E6-Ribeirão da Ilha, E7-Lagoinha do Leste, E8-Massiambu River, E9-Ratones River, E10-Sai-Mirim River.

In order to assess the responses of *L. acuta* to environmental conditions, three sites were randomly established in shallow waters at the mouth of five urbanized and five non-urbanized estuaries. The mouths of the estuaries were chosen for sampling because they sink the continental material and might therefore represent the general health of these small systems. At each site, individuals of *L. acuta*, sediment and water were sampled. The effects of urbanization on different levels of biological organization of the polychaete were assessed using: (i) the frequency of micronuclei, at the molecular level; (ii) the body size and biomass, at the individual level; and (iii) the production-to-biomass ratio (P/B), at the population level.

2.3.1 Molecular level

At each site, multiple individuals of *L. acuta* were carefully and manually collected using a spatula and tweezers, and held in acontainer with sediment and water from the local source. In the laboratory, all containers were maintained with aeration. Then, we randomly chose 10 alive and unharmed organisms (no breakage during handling) from each sampling site to evaluate the frequency of micronuclei in cells. The posterior segments of alive individuals were removed using a scalpel and were kept for four days in aquarium (natural variability of light and temperature) receiving dehydrated brine shrimp (*Branchipus stagnalis*) as food. At the end of this period, the regenerated tissues were removed, disaggregated into acetic acid and methanol at a ratio of 3:1 and centrifuged at 900 rpm for four minutes. The bulk of the material resulting from the disaggregation of the 10 specimens was fixed in methanol and stained with May-Grünwald Giemsa 5% and diluted in phosphate buffer for 10 min. Micronuclei frequencies were evaluated under a light microscope (Olympus CX21SF1, 100×magnification) by counting one thousand cells per site (bulk of 10 individuals). Micronuclei were considered as the corpuscles of smaller diameters, entirely separated and which were similar incolor and morphology to the main nuclei. The micronuclei test was adapted from Fenech (2000).

2.3.2 Individual level

From the container, we randomly select 10 other alive and unharmed organisms from each sampling site to measure for individual size and biomass. The body length was taken from the anterior prostomium to posterior pygidium of each individual under a Zeiss optical stereoscope (Discovery V12; 10 µm precision) coupled to a digital camera (AxioCam MRc 5). Additionally, the wet weight of each individual was measured (BEL Analytical balance; 0.0001 g precision and internal calibration M124AI 24 V 550 mA).

2.3.3 Population level

The biomass values of sampled individuals from each site were used to calculate empirically the production-to-biomass ratio of the population. We used the following formula adapted from the model of Brey (2012):

$\log10\ P/B = 7.947\ (-\ 2.294\ \log10M - 2409.856 \times (1/(T + 273)) + 0.548 + 582851 \times (\log10M \times (1/(T + 273)))$

Where, P=production, B=mean population biomass, M=mean individual biomass (we used a global data bank on body composition of aquatic organisms to get the energy content (Joule) per mg of wet mass; Brey et al., 2010), and T=mean seasonal temperature (we used the historical, from 1960 to 2015, mean temperature value of 17.6°C from the sampled months for all sites; INMET (2016). The value 0.548 is a product of the adapted model, since all organisms belong to Annelida (taxon=1), are infaunal (mobility=1), and occurin intertidal habitats (depth=0 m).

The population P/B ratio is the renovation or replacement of biological material, thus being the quantity of matter and energy potentially available for higher trophic level. The empiric modelof Brey (2012) used to calculate population productivity was built using sample-based data and was originally treated as a scale of area (mg/m2). In order to validate the performance of the empirical model using individual-based data (i.e., sampling a number of individuals in a population instead of sampling individuals of the population living in a specified area), we used previously published information on *L. acuta* population (Omena and Amaral, 2000). The data set is made of two year- long monthly samples and with *L.acuta* populations structured in four different cohorts. We estimated the P/B ratios based on mean individual biomass (mg) and based on mean individual biomass per square meter (mg/m2). The comparison between the P/B ratios calculated with sample-baseddata and individual-based data were found to be not significant (ANOVA, $F_{(1,40)}$= 0.080; P=0.778). We therefore assumed that aproduction-to-biomass ratio using individual-based data could beused to represent population level test in our study.

2.3.4 Environmental data

At each estuary and site, samples of near-bottom water were collected in 500mL polyethylene bottle, previously cleaned by immersion in 50% HCl, washed with deionized water and kept refrigerated at 4°C. Determination of total nitrogen, total phosphorus and total phenols was performed by spectrophotometry according to Standard Methods (APHA, 1998), using a UV visible (FEMTO spectrophotometer 700 Plus). For the particle size analyses, we used homogenized sediment sub-samples of a standardized weight (50 g). The sand fraction was assessed by dry sieving using meshes among −1.5 and 4.0 Phy, and fine fractions were accessed via pipetting at 20°C (Suguio, 1973). The aluminum, cadmium, chromium, copper and lead contents in sediments were obtained by contrAA 600 high resolution continuum source atomic absorption spectrometer (HR-CS GF-AAS, Analytik Jena AG, Jena, Germany) with a transversely heated graphite tube atomizer. The samples digestions were preceded with addition of 2.5 mL HNO3 67% and 7.5 mL HCl 37% for approximately 3.0 g, using a TOPwave IV laboratory microwave digestion system (Analytik Jena AG, Jena, Germany). The standard solution of Al, Cd, Cr, Cu and Pb were prepared from stock concentrate solution containing 1000 mg L^{-1}(Merck, Darmstadt, Germany) with nitric acid were used to prepare the external calibration curve. Additionally, to provide a basis for asserting the absence of sediment toxicity (as expected for non-urbanized estuaries) we contrasted the metal concentrations found with the Consensus-Based Threshold Effect Concentration (TEC; MacDonald et al., 2000). Similarly, to asserting sediment toxicity (as expected for urbanized estuaries) we contrasted the metal concentrations found with the Consensus-Based Probable Effect Concentration (PEC; MacDonald et al., 2000).

2.3.5 Data analysis

In order to assess the relationship between samples of the biomarkers at multiple biological scales of the polychaete and samples of environmental conditions of estuaries, a distance-based redundancy analysis (dbRDA; Legendre and Anderson, 1999; McArdle and Anderson, 2001) was used. In this analysis, the axes of ordinations are linear combinations of the environmental variables that best explain the biological variation. The Bray-Curtis coefficient and Euclidean distance were used on squared-root transformed biological and environmental data, respectively. To model the changes in each of the biological variables representing different biological organization levels (micronuclei frequency, body size, biomass, and P/B ratio) related to the environmental conditions, we used a canonical analysis of principal coordinates (CAP; Anderson and Wills, 2003). The CAP analyses how well the multivariate environmental data can predict the position of samples along a continuous values of each biomarker. CAP avoids overparameterization (i.e., to avoid including too many axes and finding spurious relationships) by choosing the number of principal coordinate axes that minimize a leave-one-out residual sum of squares (Anderson and Robinson, 2003).

Additionally, the biological and environmental variables were individually evaluated using hierarchical nested analyses of variance. The factor "condition" was fixed (two levels: urbanized vs non-urbanized) and the factor "estuary" was random and nested within the condition factor (five levels: five estuaries within each estuary type). The three sites within each estuary provided the replicates. The nested design provides an estimate of the contribution of each estuary to the total variation among replicates within the urbanized and non-urbanized comparison. Partitioning of the variances associated with each factor permits unconfounded comparisons at any of the chosen factors (Underwood, 1997). Additionally, components of variation were calculated to estimate the percentage of variability attributed to each factor and for the residual by restricted maximum likelihood estimation (Pinheiro and Bates, 1996). Data were previously tested for heterogeneity of variance with the Cochran test and squared-root transformed whenever necessary. For the ANOVAs, we used the GAD package (Sandrini-Neto and Camargo, 2012) in the R environment (R Development Core Team, 2013), and for the dbRDA and CAP we used the PERMANOVA+ add-on PRIMER software (Anderson and Gorley, 2007).

2.4 Results

The spatial distribution of *L. acuta* samples was distinct between the urbanized and non-urbanized estuaries, while three replicates from a non-urbanized estuary appeared in an intermediate position (dbRDA with 89.6% of the explained variation; Fig. 2). Samples from urbanized estuaries were characterized by higher content of metals (lead, cadmium, and chrome) in sediment and higher concentrations of dissolved nutrients (phosphorus and nitrogen) and phenol in water. Otherwise, biological samples of non-urbanized estuaries were more related with sandy sediments and well sorted grains (low variance). Nevertheless, the intermediate group of non-urbanized samples appeared to group with content of copper and very poorly sorted grains (large variance).

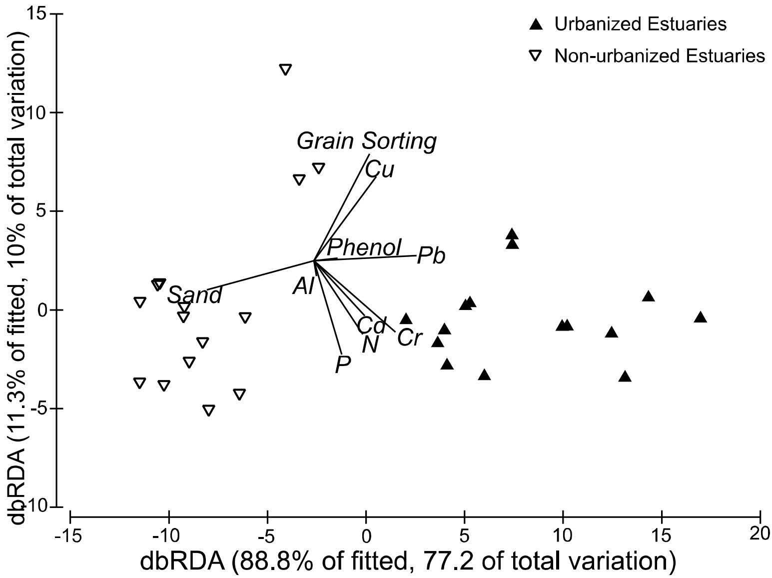


Fig. 2. Distance-based RDA ordination relating sediment and water parameters to *L. acuta* responses (micronuclei frequency, body size, biomass, and P/B ratio) in urbanized and non-urbanized estuaries.

All recorded metal concentrations in non-urbanized estuaries were lower than the Consensus-Based Probable Effect Concentration (PEC) and just one estuary (estuary 6) reported higher concentrations than the Consensus-Based Threshold Effect Concentration (TEC), indicating an absence of sediment toxicity (Fig. 3). Conversely, the chromium, copper, and lead concentrations in all sites of urbanized estuaries (except estuary 5 for copper) were higher than the TEC and some estuaries (mainly for chromium) were higher than the PEC, indicating the presence of sediment toxicity.

The analysis of variances of environmental variables showed significantly higher values for aluminum, cadmium, lead and sorting in sediment as well as nitrogen in water from urbanized estuaries than from non-urbanized ones (Tables 1 and 2; Figs. 3 and 4). The magnitude of the effects for all of those variables presented the greatest percentage of explanation related to the factor condition (all components of variation >50%). Neither environmental variable showed higher values in non-urbanized thanurbanized estuaries and the percentage of sand, content of copper and chrome in sediment, and concentration of phosphorus and phenols in water were not different between the two estuary conditions. The lowest values of components of variation (between 27and 42%) highlighted the low importance of factor conditions forthese variables. Contrarily, all variables from sediment and waterwere significantly different between estuaries nested within con-dition. This means that the variation within a group of estuaries isdifferent in general to the variation within the other group of estu-aries. In general, the urbanized estuaries were more heterogeneousamong themselves than non-urbanized estuaries (mean and errorin Figs. 3 and 4). The magnitude of the effects of the factor estuarynested in factor condition was low when the factor condition for thesame variable was significant (between 23 and 33%) and increasedonly a little when the factor condition was not significant (between34 and 51%), indicating the low importance of the variation within groups of estuaries to explain all variability found in the analysis ofvariance.

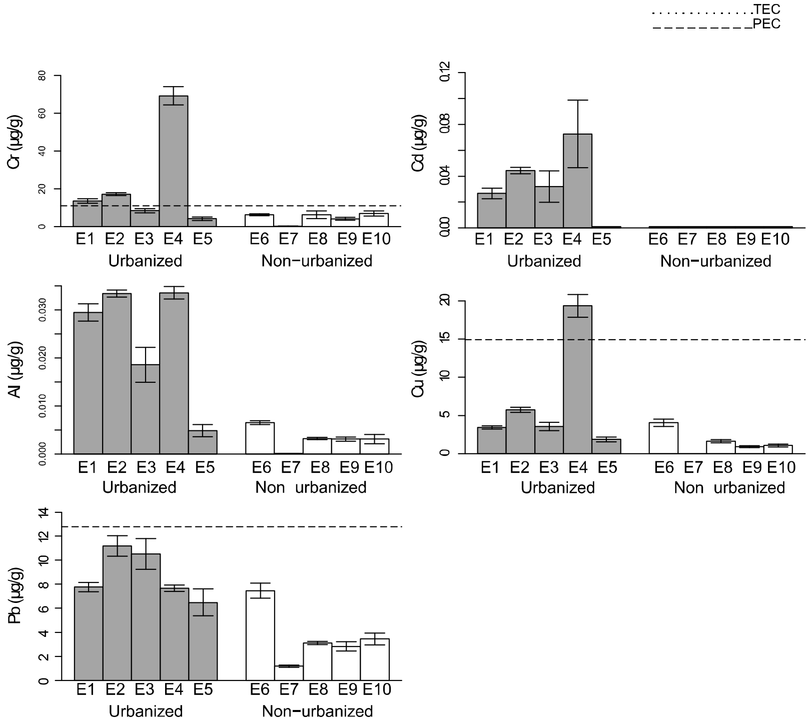


Fig. 3. Metal sediment toxicity (mean ± 1SD) in urbanized and non-urbanized estuaries. Undelines represent the Consensus_Based Threshold Effect Concentration (TEC) and the Consensus-Based Propable Effect Concentration (PEC), according to MacDonald et al. (2000). See Fig. 1 for original names of estuaries.

Table 1. Result of hierarchical nested analysis of variance and component of variation (CV) of metal from sediments in urbanized and non-urbanized (Condition) estuaries.

Source	Anova				CV (%)
	Df	MS	F-ratio	P-value	
Al					
Condition	1	0.071	15.418	**0.004**	57.038
Estuary(Condition)	8	0.005	34.818	**<0.001**	33.103
Residuals	20	<0.001			9.859
Cd					
Condition	1	0.138	12.686	**0.007**	50.059
Estuary(Condition)	8	0.011	8.806	**<0.001**	30.829
Residuals	20	0.001			19.112
Cr					
Condition	1	36.744	3.624	0.093	37.578
Estuary(Condition)	8	10.140	67.556	**<0.001**	51.490
Residuals	20	0.150			10.932
Cu					
Condition	1	12.989	4.536	0.065	41.701
Estuary(Condition)	8	2.864	91.362	**<0.001**	49.314
Residuals	20	0.031			8.985
Pb					
Condition	1	9.280	13.329	**0.006**	52.626
Estuary(Condition)	8	0.696	14.977	**<0.001**	32.375
Residuals	20	0.047			14.999

In bold $P < 0.05$, Df = degrees of freedom, MS = mean square.

Table 2. Result of hierarchical nested analysis of variance and component of variation (CV) of sediment and water parameters in urbanized and non-urbanized (Condition) estuaries.

Source	Anova				CV (%)
	Df	MS	F-ratio	P-value	
SEDIMENT					
Sand					
Condition	1	62.368	4.337	0.070	38.151
Estuary(Condition)	8	14.380	25.297	**<0.001**	45.767
Residuals	20	0.568			16.082
Grain Sorting					
Condition	1	3.765	12.778	**0.007**	49.717
Estuary(Condition)	8	0.295	7.851	**<0.001**	30.260
Residuals	20	0.037			20.023
WATER					
N					
Condition	1	0.020	16.980	**0.003**	49.880
Estuary(Condition)	8	0.001	3.211	**0.016**	23.153
Residuals	20	<0.001			26.967
P					
Condition	1	0.088	3.356	0.104	27.796
Estuary(Condition)	8	0.026	3.358	**0.013**	33.929
Residuals	20	0.008			38.275
Phenol					
Condition	1	0.010	2.473	0.155	26.587
Estuary(Condition)	8	0.004	9.900	**<0.001**	46.446
Residuals	20	<0.001			26.966

In bold $P < 0.05$, Df = degrees of freedom, MS = mean square.

Table 3. Result of hierarchical nested analysis of variance and component of variation (CV) of *L. acuta* different levels of biological organization in urbanized and non-urbanized (Condition) estuaries.

Source	**Anova**				**CV (%)**
	Df	**MS**	**F-ratio**	**P-value**	
Micronuclei frequency (1000 cells^{-1})					
Condition	1	11.981	13.837	**0.005**	54.195
Estuary(Condition)	8	0.866	20.948	**<0.001**	33.006
Residuals	20	0.041			12.800
Body size (cm)					
Condition	1	1.778	35.095	**<0.001**	59.456
Estuary(Condition)	8	0.051	3.387	**0.013**	19.114
Residuals	20	0.015			21.430
Biomass (mg)					
Condition	1	7.783	66.136	**<0.001**	66.537
Estuary(Condition)	8	0.118	3.013	**0.021**	15.069
Residuals	20	0.039			18.394
P/B ratio (y^{-1})					
Condition	1	17.115	26.764	**<0.001**	60.415
Estuary(Condition)	8	0.639	10.422	**0.013**	25.306
Residuals	20	0.061			14.279

In bold $P < 0.05$, Df = degrees of freedom, MS = mean square.

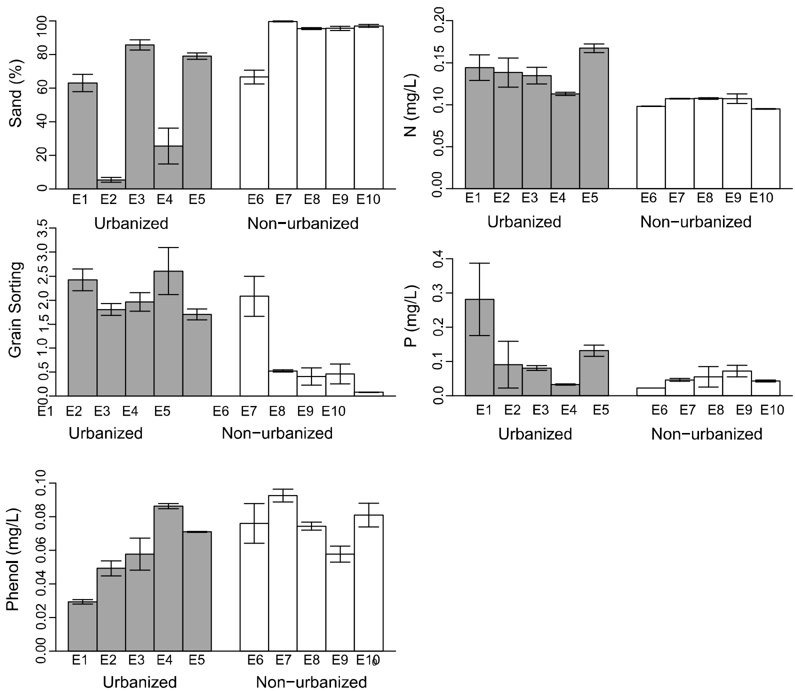


Fig. 4. Sediment and water variables (mean ± 1SD) in urbanized and non-urbanized estuaries. See Fig. 1 for original names of estuaries.

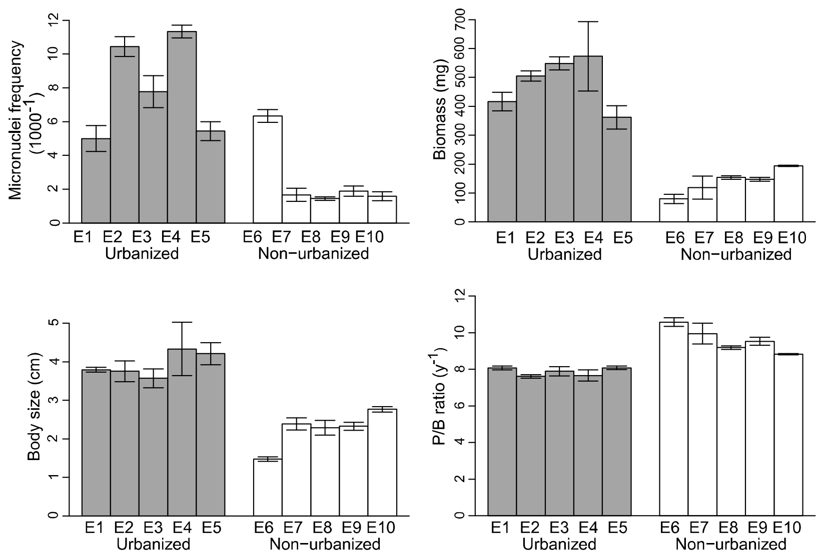
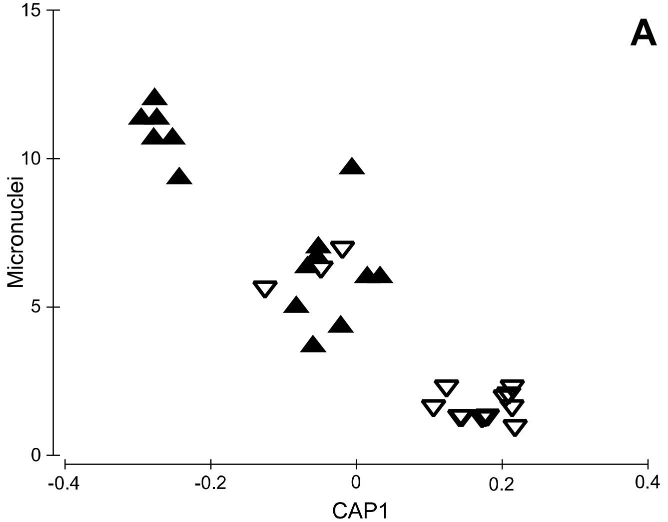
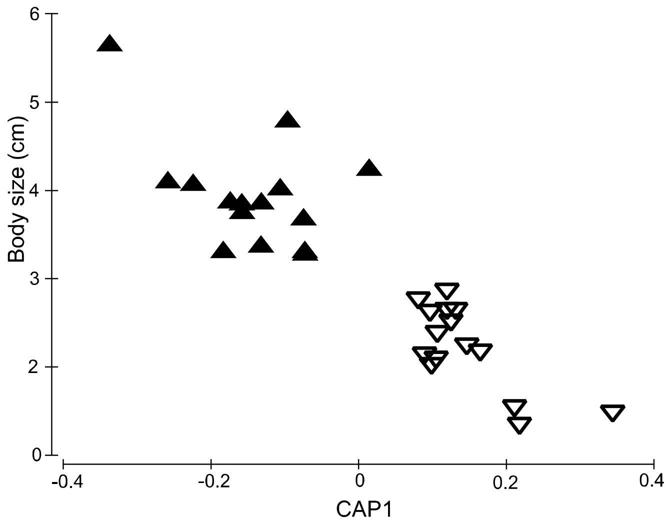


Fig. 5. Biomarkers of *L. acuta* diferente levels of biological organization (mean ± 1SD) in urbanized and non-urbanized estuaries. See Fig. 1 for original names of estuaries.

All parameters presenting the different levels of biological organization of *L. acuta* were significantly different between the conditions urbanized and non-urbanized, and among the factorestuaries nested in conditions (Table 3; Fig. 5). The lower levelsof biological organization (frequency of micronuclei at molecularlevel; body size and biomass at individual level) showed higher values in urbanized than in non-urbanized estuaries. Contrarily, thehigher level of biological organization assessed, P/B ratio of popu-lation, was significantly higher in non-urbanized estuaries than inurbanized ones. The magnitudes of the effects of the factor con-dition were always high (between 54 and 66%) and of the factorestuary were always low (between 15 and 33%). These values of component of variation indicate that contribution of the variationwithin groups of estuaries is less important for the total spatialvariability found than the variation between the urbanized and non-urbanized condition.

The CAP analyses found a strong linear relationship among each *L. acuta* biological parameters and the sediment and water properties representative of the urbanized and non-urbanized estuaries (Fig. 6). The squared canonical correlation was high and positive for micronuclei frequency (δ^2=0.86), body size (δ^2=0.79) and biomass (δ^2=0.93), and high and negative for the P/B ratio (δ^2=0.82). Additionally, the CAP analysis for micronuclei frequency showed three replicated sites of the non-urbanized estuaries were intermediately grouped among sites of the urbanized estuaries.





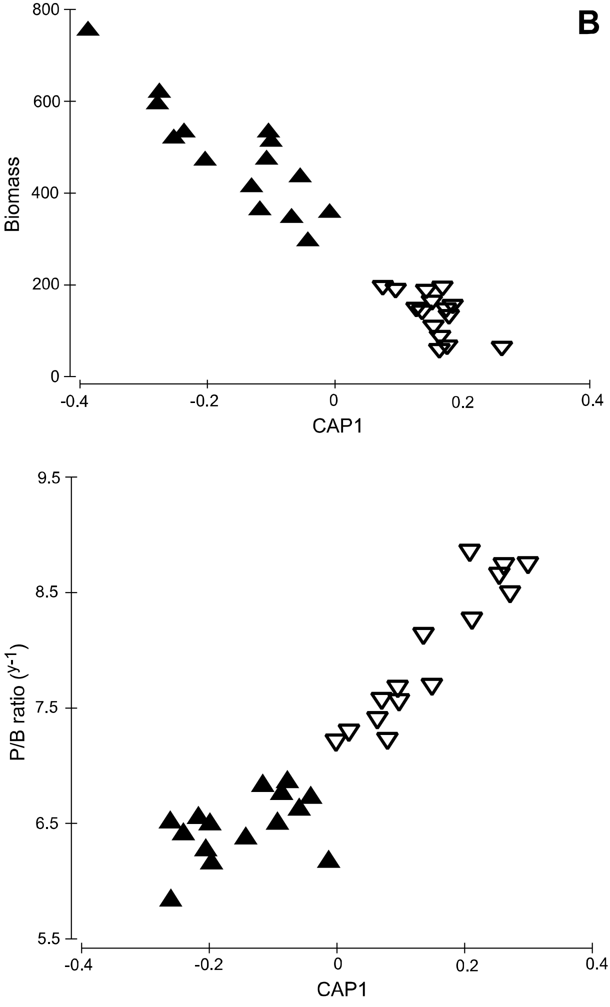


Fig. 6 (A-B). Canonical analysis of principal coordinates (CAP) to model the relationhips of sediment and water variables to *L. acuta* responses (micronuclei frequency, body size, biomass and P/B ratio) in urbanized and non-urbanized estuaries.

3. Discussion

L. acuta had significantly varying positive and negative feed-backs between urbanized and non-urbanized estuaries at multiple biological scales. These results indicate that the biomarkers showed the differences in environmental health of the studied estuaries in southern Brazil quite well. In fact, the environmental conditions of urbanized estuaries were exceeding the consensus-based threshold effect concentration (TEC) and in some sites even surpassed the consensus-based probable effect concentration (PEC). Conversely, non-urbanized estuaries never reached the PEC levels and rarely surpassed the TEC levels. Then, we confirm our hypothesis that biomarkers can detect environmental health diferences between estuaries types in all levels of biological organization studied. The detected generalized effects in all biological organization scales indicate a pollution impact on *L. acuta*. The population of the pollution tolerant polychaete is growing more, the individuals are increasing in body mass and body size, but the molecules are standing damaged. Furthermost, there is a comprehensive field investigation stressing changes in estuarine soft-bottom communities connected with changes in *L. acuta* population, expressed asincreased abundance, size and biomass (Souza et al., 2016; Pagliosaand Barbosa, 2006; Souza et al., 2013; Brauko et al., 2015; Gusmãoet al., 2016; Magalhães and Barros, 2011). On the other hand, there is a collection of laboratory experiments evidencing changes in *L.acuta* behavior, histology (coelom obliteration, separation of the cuticle from epidermis), tissue (loss of the digestive epithelium) and biochemical parameters (catalase, superoxide dismutase, lipid peroxidation, and glutathione S-transferase) caused by diferente pollutants (Geracitano et al., 2002; Ferreira-Cravo et al., 2008; Leão et al., 2008; Ventura-Lima et al., 2011). All of these evidences evoque two questions about *L. acuta* responses to pollution: (i) at the higher scales of biological organization (from individual to community), the polychaete has been efficiently occupying niche space which is vague for other species that are more sensitive to pollution. The advantage comes mostly from changing phenotypic expressions related with body size and biomass. As found in other invertebrates (Hoffmann et al., 2004), these changes in size and biomass could be rooted in the increased *L. acuta* chromosome numbers (2n = 38; ~ 36% more than others nereidids) and the consequent increased potential for chromosomal mutations related to inversions (4-fold more nucleoli per interphase cell as in other nereidids) (Ipucha et al., 2007). In fact, *L. acuta* is so different to other family speciesin many aspects that it stands in a separate and unresolved clade in phylogenetic analysis of the nereidids (Santos et al., 2006); (ii) at more basal levels of biological organization (from molecule to organ) the species developed strategies to deal with excessive pollutants and toxins. The substances are accumulated in regions ofthe body, tissues and cells, and mucus appears to be a key substance for primary protection, favoring the biotransformation of contaminants (Leão et al., 2008). The resultant permanence and resistance of the enlarged organism with accumulated contaminants in their body are eyecatching prey to consumers (Ieno et al., 2000), which might cause a bottom-up pollution transfer (biomagnification) along the estuarine food web.

The response mechanisms of organisms to pollution are usually rapidly and evidently observed at molecular and cellular scales but become rather difficult to define at higher levels due to evolutionary distance and environmental complexity (Smolders et al.,2004; Sulmon et al., 2015). Here, we detected organisms with micronucleus frequency, a type of DNA damage, three times higherin urbanized than in non-urbanized estuaries, indicating general genotoxicity of metals in the former (Sanchez-Galan et al., 1999; Ayllón et al., 2000; Andrade et al., 2004). Further, we recorded *L. acuta* DNA damage but no individual or population changes in the sites of one estuary typified as non-urbanized and presenting non-expected great concentrations of lead, copper, chromium and aluminum in sediments, indicating an early stage of genotoxicity. Metals exert genotoxic action alone or through synergistic action. In sub-lethal concentrations and chronic form, they could cause marked changes in antioxidant enzymes, suggesting the induction of oxidative stress with implications for cell repair systems (Livingstone, 2003; Nusetti et al., 2005) which affects the DNA molecule (Meneghini, 1997). Among all types of DNA damages, strand break and the subsequent micronucleus formation is the most challenging to the cells once the continuous formation of such breaks may contribute to genomic instability (Halazonetis et al.,2008).

Estuarine species living in urbanized areas are usually characterized by small organisms with low individual biomass and r-strategists (Elliott and Quintino, 2007). *L. acuta* is a typical r-strategist species with no parental care, and the presence of females with large mature oocytes and recruits throughout the year (Omena and Amaral, 2000; Santos et al., 2006; Martin and Bastida, 2006). Conversely, in the present study, the size and biomass of *L. acuta* individuals at urbanized estuaries were two-to three-fold higher than those of the non-urbanized. Possibly, the exposure of organisms to sublethal amounts of xenobiotics triggers adaptive responses providing greater ability to tolerance (Klerks and Weis,1987; Adams, 2005). Polychaetes advantages of an increased size could come from the concentration of metals in the tissues tending to a decrease in the increased sized and age of organisms (Mendezand Páez-Osuna, 1998), meaning that larger organisms are more likely to successfully thrive under contamination. When subjected to low to moderate concentrations of xenobiotics, *L. acuta* respondsby positively activating detoxifying enzymes in sufficient quantities to prevent damage caused by oxidative stress (Geracitanoet al., 2004a,b). Additionally, the production of mucus substantially increases the antioxidant defense system (Moraes et al., 2006). Thus, living in polluted environment requires many metabolic responses that demands high-energy expenditure to organisms (Bayne et al., 1979; Smolders et al., 2004). In addition, the polychaete may take advantage strategy for surviving in areas with anincreased organic material in sediments. Hence, for *L. acuta* population this excessive energy might be required to increase body size and biomass coupled with activating responses related, especially, to the antioxidant defense mechanisms. As a consequence ofenergy allocation to tolerate the environmental pollution in urbanized estuaries, the production to biomass ratio of *L. acuta* tended to decrease. That is the contrary to the expected to an opportunist species, when usually populations high density and biomass in polluted sites are supported by individuals with smaller size ranges and higher production values with a turnover ratio higher than in non-polluted sites or when pollution was ended (Alla et al., 2006; Méndez et al., 1997). All presented evidences in molecular, individual and populations ascertain *L. acuta* as a tolerant species, instead of an opportunist, and is a useful indicator of environmental pollution in estuaries.

Our results showed an environmental pollution impact in all levels of biological organization of *L. acuta* in urbanized estuaries, which indicate that even in non-heavy polluted estuaries, thes ynergisms of diffuse contaminants could cause a generalized biological effect. The main response in higher biological organization levels of the polychaete (above individual level) appears to be changes in their phenotype expression related with increased bodysize and biomass, and the consequent decreased P/B ratio. However, what appears to be a competitive advantage at higher biological levels is high cost at lower levels (bellow individual level), with DNA damage. We detected the effects of contaminants on molecular levels occur earlier, which might favor decision making by managers and stakeholder as preventive practices before the emergence of damages to populations or communities levels. All these life history characteristics of *L. acuta* point to a tolerance to pollution performance, which is a desirable feature for a useful bioindicator.

The approach used here to track the effects of contaminants at multiple biological scales using a framework of biomarkers showed accurate when contrasted with the environmental data. All used biomarkers are friendly and easy to use. The micronuclei frequencyis a lower cost and more time efficient to use than other traditional molecular approaches, as proteomic ou metabolomic. The biomarkers at individual level are widespread used and do not need any additional material than the already used in benthic ecology. The empirical population P/B ratio is a powerful tool already implemented, but unfortunately scarcely used. All used biomarkers seem to be particularly effective to highlight differences in environmental health and could be an alternative method to determine estuarine pollution condition.

Acknowledgments

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CAPÍTULO 2

Energy trade-off between somatic growth and reproduction in *Laeonereis acuta*

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ABSTRACT

The organisms' ability to obtain and transform energy can result in somatic or reproductive growth. Energy flow and quantity of resources available to higher trophic levels is an important type of ecological interaction. Environmental conditions and genetic characteristics of organisms are also importante factors for the species success or failure. Estuarine regions are naturally stressful habitats for many species and the release of anthropogenic substances increases this complexity. The objective of this study was to analyze the energetic allocation strategies using population parameters of *Laeonereis acuta* along an enrichment gradient in six estuaries of Southern Brazil. Our results show a higher longevity and adult production in the populations from urban environments. In the non-urban estuaries the organisms are smaller, with higher growth (K), mortality (Z) and young production rates. These results indicate that in changing environmental resource gradientes the polychaete *L. acuta* is able to thrive by means of energetic trade-offs between growth and reproduction.

1. Introduction

The organisms' fitness can be defined as the rate that environmental resources, in addition to those required for somatic growth and maintenance, can be obtained and used for reproduction, in other words as the ability to transform energy into reproductive effort (Brown et al., 1993). In this sense, reproductive effort can be in turn defined as the relative amount of energy invested in germ tissue production over a defined period of time (Havenhand and Todd, 1989). Many organisms have their somatic and reproductive rates reduced when facing a resource decrease, maintaining only survival rates. The nutritional status is therefore responsible for the expression and functioning of metabolic paths that will allow the animal to express its productive and/or reproductive potential (Giacomini and Shuter, 2013, Conover et al., 2009, Shuter et al., 2005, Zera & Harshman et al., 2000). The somatic production is the amount of matter or energy potentially available as food for the next higher trophic levels. Energy flow is an important type of ecological interaction, and the organism´s abilities to allocate energy between reproductive investment or somatic growth becomes a central issue in ecology. In this sense, energy becomes the currency between the interactions of any individual and its abiotic environment. However, the genetics or frequency of evolutionarily acquired alleles also plays important roles in the success or failure of species. Thus, accumulated energy and acquired phenotypes are approaches to understand underlying mechanisms (Olive et al., 2000).

Estuaries are resource-mating zones between the mainland and the ocean, naturally stressed due to the high degree of daily variability in their physical and chemical characteristics. The biota of these ecosystems is in general well adapted and largely productive, important for the maintainance of many species of fish, invertebrates, birds, and others (Elliott and Quintino 2007). However, the urbanization of coastal areas linked to nutrients or toxic effluents transported by rivers from either remote and nearby areas is a problem for sensitive species, increasing the levels of stress in these environments (Kennish, 2002, Dauvin and Ruellet, 2009). In addition, other factors may interact with the abiotic component of these sites, including marine forces, the bottom slope, sedimentary matrix, bioturbation by local fauna, etc. Among all these factors, the organic matter content can represent positive inputs and interact expressively with tolerant organisms. Interactions between organisms and resource sources can directly affect the population parameters of species, especially in the use of resources for somatic growth or reproduction (Lecerf and Richardson, 2010). In resource-constrained environments, the energy investment for one activity makes them unavailable to another.

Thus, it is ecologically important to understand how trade-offs can occur between the allocation of resources for reproduction and growth (Fischer 1930, Gadgil and Bossert, 1970). Energy allocation studies have been carried out for different organisms (Rijnsdorp, 1990, Pörtner et al., 2004). In nereididae, a semelparous family (Eckelbarger, 1994), the environmental influences can control the reproductive processes and larvae production (Olive et al., 2000). However, life history and evolutionary factors may also play a role in these reproductive strategies (Olive et al., 2000, Golding, 1994).

The nereid *Laeonereis acuta* is a tolerant polychaete widely distributed in estuarine regions of the South Atlantic. In these ecosystems, they are found in high densities (Omena et al., 2012, Pagliosa and Barbosa, 2006), presenting higher biomass and body size in urbanized environments (Weis et al., 2017). *L. acuta* is an important element in the ecology of these ecosystems, constituting a massive resource for higher trophic levels (Martin and Bastida, 2006). Thus, our hypothesis is that *L. acuta,* as a tolerant polychaete, can benefit from resources availability by directing energy trade-offs between reproduction and growth. The objective of this study was to assess the strategies for energy allocation using the parameters of population dynamics of *L. acuta* along an estuarine enrichment gradient.

2. Material and methods

2.1. Study area

This study was carried out in six estuaries: Lagoinha do Leste (LLT), Rio Ratones (RTN), Rio Massiambú (MAS), considered to be non-urbanized, and Itacorubi River (ITC), Rio Maruim (MAR) and Rio Aririú, Considered as urbanized, all located in Southern Brazil, between the coordinates 27°35'S - 48°41'W and 27°9'S - 48°20'W (Fig. 1). The studied estuaries are heterogeneous environments with different extensions, composition, grain size and type, degree of occupation and contribution from urban pollutants (Weis et al., 2017). The region is generally characterized by humid subtropical climate and the tidal regime is of micromarés, with mean amplitudes of 0.15 m in the quadrature tides and 0.83 m in the tidal tides (Cruz, 1998).

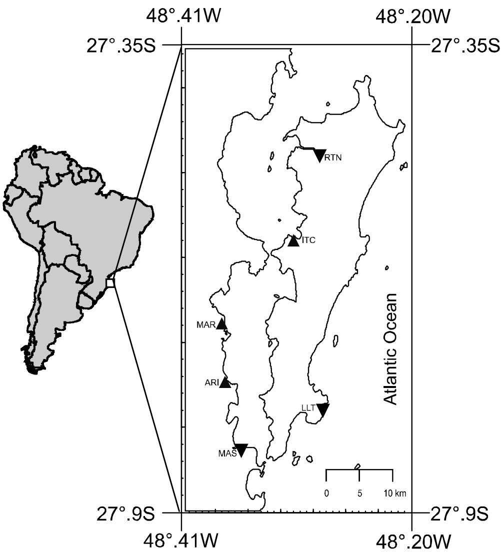

Fig. 1. Mapa indicando os seis estuários estudados na região Sul do Brasil. RTN Rio Ratones, ITC Rio Itacorubi, MAR Rio Maruim, ARI Rio Aririú, MAS Rio Massiambu, LLT Lagoinha do Leste.

2.2. Samplings

Twelve surveys were carried out in the intertidal area of each of the six estuaries during the low tide. For this purpose, six sediment samples containing fauna in three plots about 15 m apart from each other were monthly sampled from January to December 2015, with a 15 cm in diameter and 4 cm in height (0.018 m^2) sampler/corer. Three sediment samples were simultaneously taken from each site for the quantification of organic matter, using a 10 cm diameter and 4 cm high sampler (0.078 m^2).

In the field, the faunal samples were washed using a 500 µm mesh sieve, and organisms were sorted and then transferred to vials containing 4% formalin. The sediment samples were packed in ice packs and transported to the laboratory.

2.3. Laboratory procedures

In the laboratory, the fauna was identified and quantified under a magnifying glass and then transferred to 70% alcohol. The organic matter in the sediment was determined according to Suguio (1973), and the average monthly water temperature of all sites for the year 2015 was obtained through the INMET database (2016) (Annex X).

Allometric relation

In general, nereidides fragment up during the treatment of the samples, especially during screening. In this paper we use the procedure biometric based on Desrosiers et al. (1988), based on length partial and animals broken. To estimate the total length of *L. acuta*, were collected manually 443 whole individuals belonging to different size classe´s among the estuaries studied. During the collection the bodies were packed in boxes with ice and water from the samples site and transferred to the laboratory. In the laboratory, under stereoscope Zeiss optical (Discovery V12, from 270 to 480 µm precision) coupled to a digital camera (AxioCam MRc 5), were identified and taken the measures of total length (from prostomium to the pigid), the width of the 7th, 1st, 14th segments and jaw. Similarly, under microscopy was observed the cavity body and the base of parapodia of all females to identify the presence of oocytes. In addition, using the balance (analytical BEL, precision of 0.0001g and internal calibration M124AI 24V 550mA), was measured the wet weight each individual. Linear regression analysis and statistical comparision in order to find what the measure partial that best expressed the total size and weight of individuals.

The width of the 7th segment was the parameter chosen because to be best descriptor and provide the highest value of correlation with the total length. (Annex I, Annex II).

For the population dynamics of each estuary, the following developmental classes were established: Fertile females (individuals presenting oocytes), adults (individuals greater than the lowest fertile female, with absence of oocytes) and juveniles (individuals smaller than the lowest Fertile female). The total length and growth parameters were estimated using the width of the 7th setígeros. To determine the influence of the resource availability gradient on the population dynamics of L. acuta, the following parameters were used: ($L\infty$) length infinity in mm, maximum age (t_max), lowest individual (t_i), lowest fertile female (t_sex), Longest length ∞), growth constant (K), best fit value (Rn_max).

2.4. Data analisys

To reduce environmental information on a single scale representative of the resource availability gradient, the pollutants (aluminum, cadmium, chromium, copper and lead, total nitrogen, total phosphorus (detailed in Weis et al., 2017) and the steroids Cholesterol and cholestene (see Chapter 2 for details) under went a Principal Component Analysis (PCA) (Annex II).

Then, to determine the relationships between the use of the resource by *L. acuta* and the gradient of environmental stressors extracted by PCA, Kernel density curves were performed for the populations of each of the six estuaries. The objective of this analysis was to model the probability of total density and density per stage of development (juveniles, fertile females and adults) of *L. acuta* within each estuary.

For the calculation of the secondary production the Crisp method (1984) was used with the growth rate. The monthly biomass at the different stages of development was calculated by multiplying the average density of individuals in each month by the average weight of the individuals in each month. Somatic production and annual average biomass are equivalent to the sum of the production and the average biomass of the study months.

The monthly population parameters of *L. acuta* in the six estuaries were estimated using Electronic LEngth Frequency ANALYSIS. The values of L∞ were obtained from the largest size class in the population by the standard ELEFAN_GA option (variation from 0.5 to 1.5 above the matrix values) indicated by the best fit value (Rn_max). Through this routine were also obtained the parameters for life time, larger size, and growth constant, using the value of the method reliability checker (PhiL). The mortality rate was obtained through Cath_curve, a linear model in which estimates of instantaneous mortality rates (Z) are obtained.

To model the relationship between fertile females and the availability of resources, a logistic functions approach and parameter estimates were adopted de acordo com Restrepo and Watson (1991).

For the PCA, we used the PERMANOVA+ add-on PRIMER software (Anderson and Gorley, 2007) end for the population parameters for *L. acuta* in each of the six estuaries were estimated using length-frequency data in the package TropfishR (Mildenberger et al., 2017) in R software (R Development Core Team, 2016). The package added accuracy to the estimation of growth parameter (derived from the von Bertalanffy growth function) by including the improved version ELEFAN-GA to the traditional ELEFAN (Electronical LEngth Frequency ANalysis) method (Taylor & Mildenberger 2017).

3. Results

For the population trends in the Kernel curves, Fig. 1 (A) showed two situations with patterns of opposite densities along the enrichment and pollution gradient, and Fig. 1 (B) showed, in turn, an optimum gradient of resource availability in common for three different stages of development in *L. acuta*. In the most polluted situation the curves reached the probability of densities at larger ranges along the pollution axis. On the other hand, the less polluted situation presented high probability curves of densities at shorter contamination ranges. For instance, the LLT curve displayed a very small value for bandwidth and a large relative estimated density compared to the curves from the other estuaries.

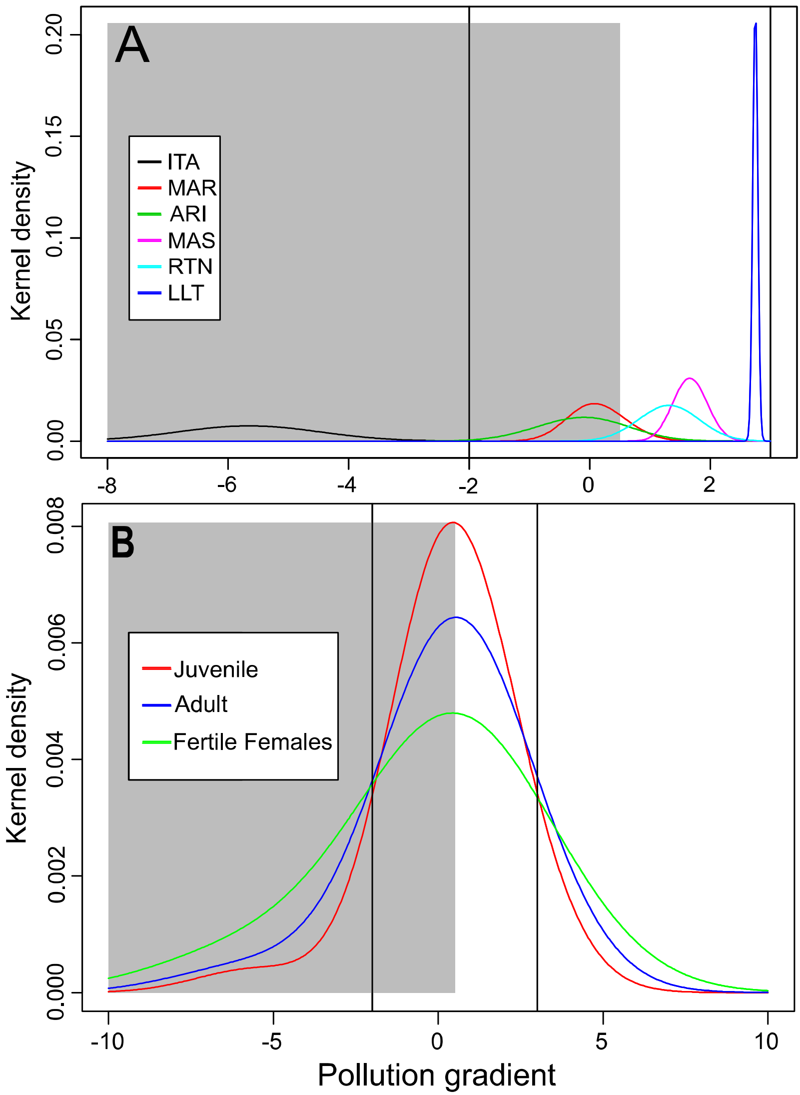

Fig. 1: The Kernel density estimator to generate a probability density distribution for *L. acuta* at different stages of development (juvenile adult and fertile females) in urbanized and non-urbanized estuaries.

The population parameters for *L. acuta* showed distinct trends along the enrichment and resource availability gradient. In estuaries with more resources or urbanized, the population parameters were of larger size of individuals, higher longevity and higher production of adults. However, in estuaries with less or no urbanization, the parameters that represented the growth factor (K), the mortality rate (Z) and the juvenile production were higher (Table 1).

Tab. 1 Growth parameters of *Electronic LEngth Frequency ANalysis* with genetic algorithm used for estimating growth parameters.

	MAS	LLT	RTN	ARI*	ITA*	MAR*
t_max	4.629	1.980	3.885	4.732	6.139	4.457
t_i	0.995	1.012	1.028	1.519	1.495	1.457
t_sex	0.517	1.005	0.926	1.421	1.243	1.060
$L\infty$	3.772	3.324	3.693	4.351	4.244	4.270
K	0.138	0.180	0.149	0.115	0.119	0.120
Rn_max	0.897	0.918	0.924	0.941	0.936	0.941

T_max, maximum age of species; *t_i*, lower individual; *t_sex*, lower female; $L\infty$, length infinity; *k*, growth constant; *Rn_max*, highest value of fitness function. Urbanized estuaries*

The lower concentration of the resource was determinant for the females to start their reproductive period sooner. In less enriched sites females invest more energy in reproduction, whereas in more enriched sites we observe that the reproductive cycle begins later, with the possibility of energy investment in growth and longevity (Fig. 2).

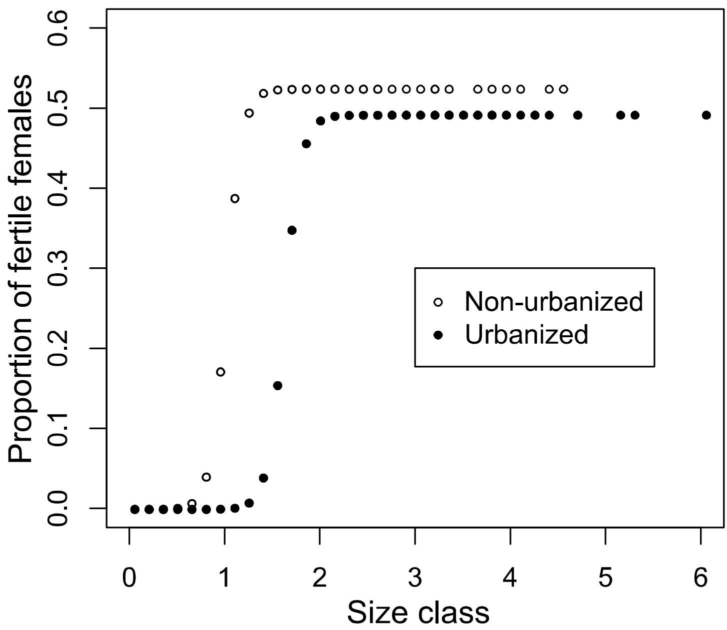

Fig. 2: Observed proportions of fertile females in non-urbanized estuaries (circles) and fertile females of urbanized estuaries (filled circles).

4. Discussion

The sediments of urbanized estuaries are modified by the presence of metals, sterols, nutrients and other substances. However, in the studied estuaries, the nature and concentration of sediment compounds of anthropogenic origin in fact represent a source of resources and opportunities for the species *Laeonereis acuta*. This species is tolerant to this pollution scenario and has demonstrated physiological abilities to colonize these environments (Leão et al., 2008), being found in high population densities even under pollution stress (Martin and Bastida 2006). For more sensitive species, these effects may be deleterious and imply compensatory energy costs (Freitas, 2017, Dafforn et al., 2012, Wang et al, 2010).

Studies with macrophysiological approaches show that subpopulations of the same species may differ along large latitudinal or climatic scales (Calosi et al., 2017, Conover et al., 2009, Gaston et al., 2009). In the present study we identified distinct pattrerns in subpopulations of *L. acuta* living in estuaries in the same region and separated by small distances, from one to few tens of kilometers, that might be linked to physiological patterns. In this estuarine gradient we found opposite values for L∞, growth constant (K), longevity, mortality (Z), young production and adult production. In all estuaries, the organisms showed a period of higher growth and another of lower growth, but the general growth pattern in more enriched urban sites were the highest, such differences being related to the environmental resource availability gradient.

In this study we found that the highest number of fertile or ovigerous females in all studied estuaries occurred when average air temperature was generally lower. In colder periods, the general metabolism of these ecosystems decreases (Renaud et al., 2015), coinciding with lower primary production and more stable conditions (Jumars and Wheatcroft, 1989). The lower temperature stations can then be considered stations where the accumulated energy of the animals is directed to reproduction. Although there are divergences about the importance of this parameter (Santos et al., 2002), the effects of temperature on egg production in other invertebrates is already an old observation (Wolda, 1964).

Previous studies have demonstrated that *L. acuta* can be found in high densities in urbanized sites (Omena and Amaral, 2000; Pagliosa and Barbosa, 2006), as an important contribution to the total productivity of this ecosystem. Thus, the different patterns found in this study are more likely to be correlated to the effects of the presence of anthropogenic substances in the sediments of the estuaries, as an increase in available resources. *L. acuta* might use such resources by allocating energy for higher growth and longevity. In contrast, in non-urbanized estuaries with less organic matter contents, *L. acuta* might allocate exceeding energy towards reproduction. The populations of these estuaries showed reproductive pulses with a large number of juveniles, and adults are more rarely found. These results indicate that the resource gradient influences the trade-off between energy allocation between somatic and reproductive growth in *L. acuta*.

The overlap of genetic factors to environmental factors has been discussed in Levins (1968, 1969), and the physiological responses of *L. acuta* to exposure to moderate concentrations of xenobiotics are also known (Moraes, et al., 2006, Geracitano 2004a, 2004b). Nutrition is known to have a strong effect on fertility in many organisms (Horwood, 1989). When subjected to an environmental enrichment gradient, *L. acuta* expresses its abilities by allocating its energy stocks for growth and longevity instead of juvenile production. Our results suggest that the quantity of resources directly influence the population dynamics of *L. acuta*. Thus, the role of extrinsic (eg, nutrition and climate) and intrinsic (eg, genetic or adaptive) factors at a spatial microscale can influence the performance and behavior of individuals or populations (Petchey 2006, Lange et al., 2016).

As shown in Weis et al., (2017), *L. acuta* presents two to three times larger body sizes in urbanized sites, using the empty niche left by sensitive species, the available resources, and presenting tolerance to xenobiotics. Allometric relationships and availability of resources have become important ecological approaches to explain the population dynamics of the species. (Maurer et al., 1992). In addition, size is a key feature and one of the most important attributes in ecology, affecting biological processes at all levels of organization, from cellular metabolism to population dynamics (Calder, 1984, Etienne et al., 2012). This feature is also correlated with many aspects of life history, like metabolic efficiency, generation time, metabolism, reproduction rate and dispersion (Peters, 1983).

The current tolerant status of *L. acuta* and the consequent effective use of available resources in urbanized environments increases the survival probability and influence the type (quality of phenotypes) of individuals entering the population in subsequent generations. Well-developed recruits result in more fertile adults with higher reproductive probabilities (Burgess and Marshall 2011). Thus, the effects of past conditions, especially parental conditions, still drive present generations, influencing the life history of present itself and future generations (Beckerman et al., 2002). In this way, the present populations are influenced by the interactions between the past habitat of colonizors and the status or quality of the current habitat. Our results suggest that the behavior of *L. acuta* and its ability to use resources in urban environments along with changes in body size can be useful parameters to estimate the effects of anthropogenic pressures, and could be included in real-world current programs of environmental monitoring.

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MATERIAL SUPLEMENTAR

Anexo I

Relação alométrica

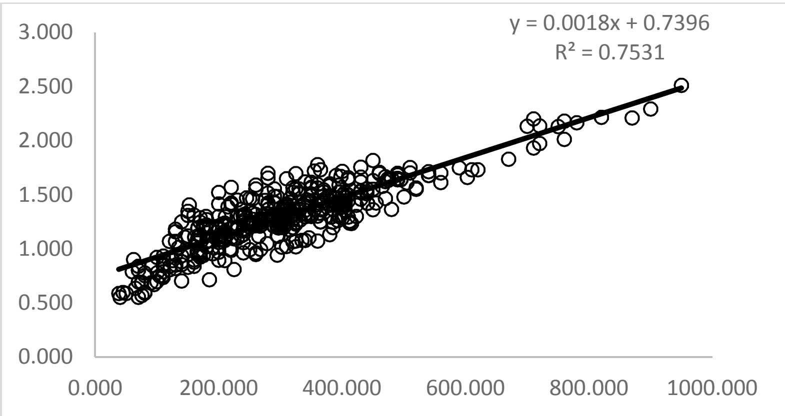

Fig. 2. Allometric correlation between a width of 7th setígeros and the total length of *L. acuta*.

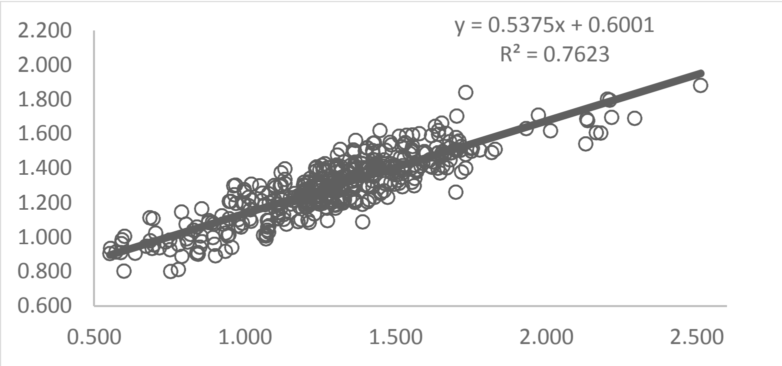

Fig. 2. Allometric correlation between the width of the 7th setígeros and the biomass of *L. acuta*.

AnexoII

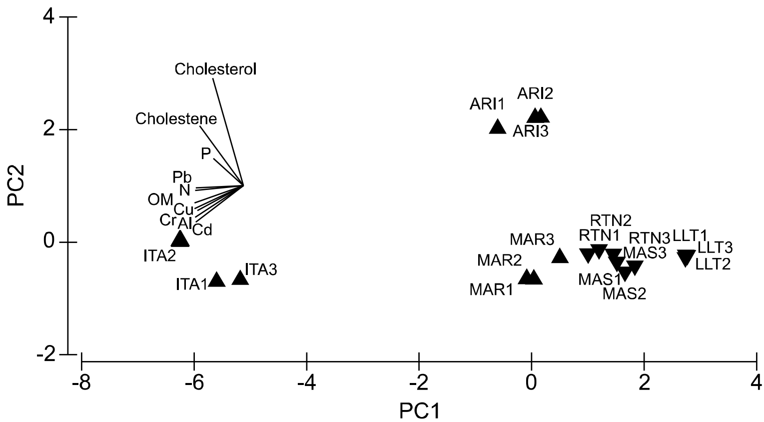

Fig. 3. *Principal Component Analysis* (PCA) evidencing the pollution gradiente along the estuaries. Chemical variables were equationed by bulk density of sediment samples and correlated to the distribution of sampling plots (triangles). Urbanized estuaries: ARI, ITA, MAR; Non-urbanized estuaries: MAS, LLT, RTN. Proportion explained: 78.4% for PC1 and 10% for PC2. P = phosforous; N = nitrogen; OM = organic matter; Pb = lead; Cu = copper; Cr = chrome; Al = aluminum; Cd = cadmium.

6 TABELAS

Anexo III

Tab. I: Temperatura média mensal da água dos estuários estudados, durante o ano de 2015.

Local	Date	Water_temp
MAS, LLT, RTN, ARI, ITA, MAR	January	23.394
	February	26.343
	March	25.741
	April	23.748
	May	22.39
	June	20.557
	July	18.503
	August	20.207
	September	19.15
	October	19.466
	November	21.82
	December	23.909

Anexo IV

Tabela II: *Laeonereis acuta*. Sumário dos dados usados para cálculo da biomassa e produção secundária anual segundo o método de Crisp (1971) de janeiro a dezembro de 2015 no estuário do rio Massiambu (MAS).

MAS	midLengths	Ymd	Wmg	varY	Gmg	Biom_g	varY_g	N=10
	0.25	2729.12	0.73	NA	NA	2	NA	PROD
	0.75	3279.54	1	-550.42	0.86	3.29	-0.47	-4.72
	1.25	1137.59	1.27	2141.95	1.13	1.45	2.42	24.2
	1.75	329.16	1.54	808.44	1.4	0.51	1.13	11.32
	2.25	91.75	1.81	237.41	1.67	0.17	0.4	3.96
	2.75	20.82	2.08	70.92	1.94	0.04	0.14	1.38
	3.25	6.84	2.35	13.98	2.21	0.02	0.03	0.31
	3.75	1.52	2.62	5.32	2.48	0	0.01	0.13
	4.25	5	2.88	-3.48	2.75	0.01	-0.01	-0.1
	4.75	0.76	3.15	4.24	3.02	0	0.01	0.13
Produção total anual						7.49	39.88	

Anexo V

Tabela III: *Laeonereis acuta*. Sumário dos dados usados para cálculo da biomassa e produção secundária anual segundo o método de Crisp (1971) de janeiro a dezembro de 2015 no estuário do rio Massiambu (LLT).

LLT	midLengths	Ymd	Wmg	varY	Gmg	Biom_g	varY_g	N=4
	0.25	2037.66	0.73	NA	NA	1.5	NA	PROD
	0.75	262.48	1	1775.18	0.86	0.26	1.52	6.1
	1.25	79.34	1.27	183.14	1.13	0.1	0.21	0.83
	1.75	61.7	1.54	17.64	1.4	0.1	0.02	0.1
Produção total anual						1.96	7.65	

Anexo VI

Tabela IV: *Laeonereis acuta*. Sumário dos dados usados para cálculo da biomassa e produção secundária anual segundo o método de Crisp (1971) de janeiro a dezembro de 2015 no estuário do rio Massiambu (RTN).

RTN	midLengths	Ymd	Wmg	varY	Gmg	Biom_g	varY_g	N=8
	0.25	569.99	0.73	NA	NA	0.42	NA	PROD
	0.75	277.84	1	292.14	0.86	0.28	0.25	2.01
	1.25	36.74	1.27	241.1	1.13	0.05	0.27	2.18
	1.75	14.23	1.54	22.51	1.4	0.02	0.03	0.25
	2.25	9.42	1.81	4.81	1.67	0.02	0.01	0.06
	2.75	2.28	2.08	7.14	1.94	0	0.01	0.11
	3.25	1.62	2.35	0.66	2.21	0	0	0.01
	3.75	0.76	2.62	0.86	2.48	0	0	0.02
Produção total anual						0.79	5.06	

Anexo VII

Tabela V: *Laeonereis acuta. Sumário* dos dados usados para cálculo da biomassa e produção anual secundária segundo o método de Crisp (1971) de janeiro a dezembro de 2015 no estuário do rio Aririú (ARI).

ARI	midLengths	Ymd	Wmg	varY	Gmg	Biom_g	varY_Gg	N=10
	0.25	2274.25	0.73	NA	NA	1.67	NA	PROD
	0.75	4402.6	1	-2128.35	0.86	4.42	-1.83	-18.27
	1.25	2310.49	1.27	2092.11	1.13	2.94	2.36	23.63
	1.75	1128.79	1.54	1181.7	1.4	1.74	1.65	16.54
	2.25	219.07	1.81	909.73	1.67	0.4	1.52	15.19
	2.75	22.38	2.08	196.68	1.94	0.05	0.38	3.81
	3.25	4.98	2.35	17.4	2.21	0.01	0.04	0.38
	3.75	0.76	2.62	4.22	2.48	0	0.01	0.1
	4.25	0	2.88	0.76	2.75	0	0	0.02
	4.75	0.76	3.15	-0.76	3.02	0	0	-0.02
Produção total anual						11.22	45.1	

Anexo VIII

Tabela VI: *Laeonereis acuta*. Sumário dos dados usados para cálculo da biomassa e produção anual secundária segundo o método de Crisp (1971) de janeiro a dezembro de 2015 no estuário do rio Aririú (ITA).

ITA	midLengths	Ymd	Wmg	varY	Gmg	Biom_g	varY_Gg	N=13
	0.25	194.57	0.73	NA	NA	0.14	NA	PROD
	0.75	1520.38	1	-1325.81	0.86	1.53	-1.14	-14.79
	1.25	913.43	1.27	606.95	1.13	1.16	0.69	8.91
	1.75	808.9	1.54	104.53	1.4	1.25	0.15	1.9
	2.25	392.18	1.81	416.72	1.67	0.71	0.7	9.05
	2.75	99.43	2.08	292.75	1.94	0.21	0.57	7.38
	3.25	3.9	2.35	95.53	2.21	0.01	0.21	2.74
	3.75	6.97	2.62	-3.07	2.48	0.02	-0.01	-0.1
	4.25	0.76	2.88	6.21	2.75	0	0.02	0.22
	4.75	1.15	3.15	-0.39	3.02	0	0	-0.02
	5.25	1.15	3.42	0	3.28	0	0	0
	5.75	0	3.69	1.15	3.55	0	0	0.05
	6.25	0.76	3.96	-0.76	3.82	0	0	-0.04
Produção total anual						5.03	16.68	

Anexo IX

Tabela VII: *Laeonereis acuta*. Sumário dos dados usados para cálculo da biomassa e produção anual secundária segundo o método de Crisp (1971) de janeiro a dezembro de 2015 no estuário do rio Aririú (MAR).

MAR	midLengths	Ymd	Wmg	varY	Gmg	Biom_g	varY_Gg	N=9
	0.25	4656.81	0.73	NA	NA	3.42	NA	PROD
	0.75	6998.26	1	-2341.45	0.86	7.02	-2.01	-18.09
	1.25	3459.89	1.27	3538.36	1.13	4.4	4	35.97
	1.75	1632.4	1.54	1827.49	1.4	2.52	2.56	23.03
	2.25	551.37	1.81	1081.03	1.67	1	1.8	16.24
	2.75	172.78	2.08	378.6	1.94	0.36	0.73	6.61
	3.25	38.56	2.35	134.22	2.21	0.09	0.3	2.67
	3.75	9.74	2.62	28.82	2.48	0.03	0.07	0.64
	4.25	2.28	2.88	7.46	2.75	0.01	0.02	0.18
Produção total anual						18.84	73.28	

CAPÍTULO 3

Laeonereis acuta (Annelida: Polychaeta) population dynamics and sediment fatty acids in estuaries under urbanization pressure

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Abstract

Population dynamics of indicator species can be powerful tools for environmental quality assessment while they underpin the processes shaping species life history trends and long-term ecosystem functioning. In coastal and marine habitats, the stability of benthic population trends is largely susceptible to changes in both quality and quantity of organic compounds inputs, since they process and absorb such compounds. In this sense, we assessed the population dynamics of the polychaete *Laeonereis acuta* in six estuaries under distinct levels of urbanization in the Southern Atlantic, and tested the correlations between population parameters with sediment fatty acids (used as chemical markers) from these environments over a 12-months period. Growth was calculated using computer-based methods of length-frequency data (ELEFAN-GA) and the multivariate data analyses involved a PERMANOVA and dbRDA. *L. acuta* population dynamics varied following the urbanization levels of estuaries, and were influenced by the distribution of sediment fatty acids. However, despite widely known as an opportunistic species, the population varied contrarily to r- and k-strategists expectations, as a possible overlap in adaptive life strategies and plastic physiological mechanisms of tolerance. Higher biomass, size and life span within the urban estuaries could also represent responses to local increasing inputs of hormones, either of natural or synthetic origin. Our results based on robust data interpretations highlight that besides underpinning species life history patterns that maintain ecosystem outputs and functions, the parameters of population dynamics for this polychaete may also directly indicate the environmental quality linked to anthropogenic use.

Introduction

Populations respond often in a complex and dynamic manner to changing habitats, but population dynamics is rarely considered in current approaches for environmental quality control. At a first moment, population parameters might only indicate growth, mortality and reproduction patterns, but can be powerful tools while they underpin the processes shaping species life history trends and long-term ecosystem functioning. The trends of variation in population dynamics also represent proxies for extinction risk of key species that support ecosystems goods and services (or functions). Decision makers can make full use of this data not only for environmental quality assessment, but also to more realistically account for processes such as local extirpation, demographic rescue, source-sink dynamics and dispersal limitations (Fordham et al., 2013).

In coastal and marine habitats, the stability of benthic population trends is largely susceptible to changes in both quality and quantity of organic compounds inputs. By processing or absorbing such compounds, the benthic fauna of intertidal zones is of major vulnerability for inhabiting a system that receives a relatively large fraction of organic matter from terrestrial sources. This type of organic matter might derive from natural processes (e.g. animal and plant decomposition), but the anthropogenic-derived compounds are of special concern (Zimmerman and Canuel, 2000), mainly in highly urbanized areas.

Anthropogenic impacts linked to urbanization expansion such as eutrophication, pathogens and toxic xenobiotic compounds are likely to be the most pressing problems along coastlines (Wu, 1999). Although these compounds normally occur at very low concentrations, there is growing concern about the chronic exposure and biomagnification of xenobiotics (Bolong et al., 2009), and about the kinds of chemical markers able to indicate *in situ* environmental impacts. Fatty acids have been used as biomarkers of both natural and anthropogenic processes on the distribution of organic matter from different sources in coastal sediments, by distinguishing the relative contribution of primary producers (e.g. microalgae), secondary producers (e.g. bacteria) and terrestrial inputs (Atanassova and Mills, 2016, Fang et al., 2014, Venturini et al., 2012). In addition, fatty acid biomarkers have been used as geochemical indicators of eutrophication processes (Pinturier-Geiss et al., 2015).

At present, most of our knowledge on toxic effects of xenobiotic compounds is derived from short-term exposure of a single species to highly environmental unrealistic and uniform concentrations under laboratory conditions. Data so derived are inadequate in predicting ecological effects in the field, in which the populations of indicator species in several different life stages are being exposed to varying low concentrations under an interacting and complex environment (Mons et al., 2013). There is urgent need in understanding how population dynamics of potential indicator species change under anthropogenic pressures. Polychaete species are frequently used as indicators in marine health assessments, and population dynamics of nereidid species, a particularly abundant, responsive and widespread family, have been intensively investigated (Pagliosa and Lana, 2000; Omena & Amaral, 2000; Labra et al., 2016). These polychaetes have also been suggested as a candidate target in biomonitoring protocols of estuarine areas, so an in-depth understanding of its population dynamics and its responses to urbanization pressures are in need. In special, their possible links to the abundant families of fatty acid compounds from sediments are yet to be understood.

We present herein a consistency test on real-world data concerning a bioindicator candidate for environmental quality assessment and emerging chemical compounds that might signal unnoticed hazards released from human waste. Our objectives were to assess the population dynamics of *Laeonereis acuta* in six estuaries under distinct levels of urbanization in the Southern Atlantic, and to test the correlations between a series of population parameters with sediment fatty acids from these environments over a 12-months period, using a robust multivariate approach.

Materials and Methods

Study area

The study was conducted in six estuaries from Southern Brazil between the coordenates 27°35´S-48°41´W and 27°9´S-48°20´W : Lagoinha do Leste (LLT), Ratones (RTN), Massiambú (MAS), Itacorubi (ITC), Maruim (MAR) and Aririu (ARI) (Fig. 1). These estuaries are environmentally heterogeneous among themselves, with different extensions, composition, sediment type and size of grain, degree of human occupation and inputs from urban effluents (Weis et al., 2017). The region is in general characterized by a subtropical humid climate and microtidal regime, with a tidal range from 0.15 m to 0.83 m in neap and spring cycles, respectively (Cruz, 1998).

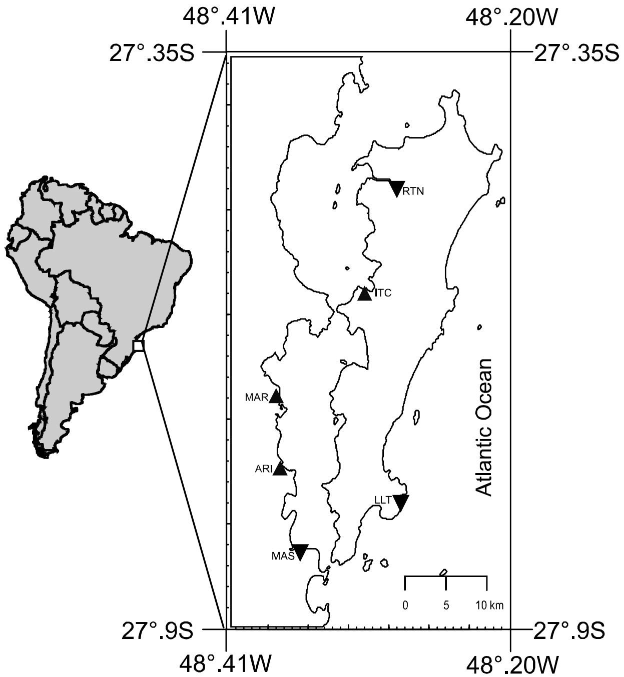

Fig. 1. Map of the six estuaries in the Southern Atlantic. Urbanized estuaries: ITC – Itacorubi, MAR – Maruim and ARI – Aririu; Non-urbanized estuaries: RTN- Ratones, MAS – Massiambu and LLT – Leste Lagoon.

Samplings

The *L. acuta* populations were monthly surveyed in 12 samplings (January to December 2015) in the intertidal portion of six estuaries, during the low tide. In each estuary, two replicates from three plots (approximately 15 m apart from each other, n = 432) were manually sampled using a PVC macrofaunal corer of 15 cm diameter x 4 cm height

(0.018 m²). Samples were washed *in situ* with a 500 µm macrofaunal sieve, and all individuals retained were fixed in 4% formalin before being transported to the laboratory.

Sediment samples were additionally taken at each plot during all surveys using a 10 cm diameter x 4 cm height corer (0.078 m²) for sedimentology (n = 216), and in the September survey one sediment sample per plot was also taken for fatty acids analyses (n = 18).

Laboratory procedures

For population dynamics of *L. acuta*, all individuals per sample were identified and counted under the stereoscopic microscope, and preserved in 70% alcohol. Then, the ovigerous females (presence of visually identifiable oocytes), adults (absence of oocytes and larger than the smallest ovigerous female) and juveniles (all individuals smaller than the smallest ovigerous female) were grouped and counted.

In general, nereids break down during sample processing, especially during sieving. In this work we used the biometric procedure based on Desrosiers et al. (1988), which includes the partial length of broken animals. Thus, to estimate the total length of *L. acuta*, 443 whole individuals belonging to different size classes were manually sampled during the surveys. After samplings, the organisms were packed in ice boxes with local water and then transferred to the laboratory. In the laboratory, under a Zeiss optical stereoscope (Discovery V12, from 270 to 480 µm precision) coupled to a digital camera (AxioCam MRc 5), we measured the total length (from prostomium to pigidium), the 7 °, 10 °, 14 ° segments and the mandible. Similarly, the body cavity and base of the parapodia of all females were observed under microscopy to identify the presence of oocytes. Additionally, the wet weight of each individual was measured using a analytical scale BEL of 0.0001g precision and internal calibration M124AI 24V 550 mA. Simple linear regression analysis were performed to test which partial measure best expressed the total size and weight of individuals. The width of the 7° segment was the chosen parameter or best descriptor, with the highest correlation value to the total length. (see regression equation in Supplementary material 1).

The sediment analysis accounted for granulometry and organic matter content following the Suguio (1973) standard methodology. For the sediment fatty acids GC-MS analysis were performed. For these determinations the samples were prepared using previously optimized and validated procedure. Briefly, 12 g of the sample was extracted using 30 ml of methyl test butyl ether (MTBE). Obtained extract was evaporated to the volume of 1 ml and transferred onto silica gel/anhydrous sodium sulfate clean up cartridge. Before clean up, the cartridge was preconditioned with 5 ml of methanol. Analytes were eluted using MTBE (10 ml), the eluate was evaporated under the gentle nitrogen stream and reconstituted with to the volume of 0.5 ml. Obtained extract was dried under a gentle N2 stream and derivatized using N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in acetonitrile to form TMS-ethers (Trimethylsilyl ethers) at 70 °C for 1h before instrumental measurements.

A Zebron ZB-5MSi GC columns - Phenomenex (USA.) was then employed in these analyses: 10 m, 0.18 mm i.d. fused silica capillary, coated with 5% phenyl 95% dimethyl polysiloxane stationary phase, 0.18 ìm film thickness for fast-GC/MS work. Helium was used as the carrier gas at a Dani Master TOF-MS (Italy) Instrument. 1.5 µL sample was injected; and the analyses conditions with oven initial temperature 50 °C, Equilibration time 1.0 min; temperature programming was at 15 °C/ min to 295 °C, with a final isothermal period of 3.0 min. Compounds were tentatively identified by the standard spectral library (NISTL) and the reported literature.

Data analysis

The population parameters for *L. acuta* in each of the six estuaries were estimated using length-frequency data in the package TropfishR (Mildenberger et al., 2017) in R software (R Development Core Team, 2016). The package added accuracy to the estimation of growth parameter (derived from the von Bertalanffy growth function) by including the improved version ELEFAN-GA to the traditional ELEFAN (Electronical LEngth Frequency ANalysis) method (Taylor & Mildenberger 2017). The von Bertalanffy growth function (VBGF) was used as follows:

$$Lt = L_\infty \left[1 - e^{\left[-k(t-to) + (KC/2\pi)\mathrm{sen}(2\pi(t-ts)) - (KC/2\pi)\mathrm{sen}(2\pi(to-ts)) \right]} \right],$$

where: Lt = length at age t; $L\infty$ = maximum asymptotic length; k = growth rate; t_0 = computed age at length zero; C = parameter reflecting the intensity of seasonal oscillation; ts = start of a sinusoid growth oscillation with respect to t = 0, and WP (Winter Point, which corresponds to the moment of lowest growth rate during the year cycle) = ts + 0.5 yr. The growth parameters were estimated in two steps: (1) preliminary estimates of $L\infty$ were obtained by the method of Wetherall (1986) as modified by Pauly (1986), and (2) this estimated $L\infty$ was the used as a 'seeded' value for fitting a growth curve to the length-frequency data – Rn or the 'goodness of fit' index - of the ELEFAN-GA routine. The higher accuracy of the routine can be attributed to the genetic algorithm implemented in ELEFAN-GA, which introduces less noise in the data simulation process. Life span was calculated from the L_{max}, e.g. the 95% of the asymptotic length ($L\infty$) in the von Bertalanffy growth curve. The length-converted catch curve method was then used to estimate the rates of instantaneous mortality (Z) per month. The growth performance index (phiL index) was computed from the equation: phiL = $\log_{10}$ K + 2$\log_{10}$ $L\infty$ (Pauly and Munro, 1984).

To test eventual differences in population parameters in relation to the distinct urbanization levels of estuaries we used a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), based on a square-root transformed Bray–Curtis dissimilarity matrix. The one-way linear model included the factor "Condition" - fixed, with two levels (Urbanized and Non-urbanized) and three replicates for each condition represented by estuaries. The multivariate faunal matrix included all population parameters (life span, initial size of individuals (S_i), size of adults (S_{ad}), asymptotic length ($L\infty$), growth performance index (phiL), growth coefficient (K), the T_{anchor}, summer point (Ts), mortality rate (Z) and biomass values.

The relationship between sediment fatty acids and population parameters were explored using Distance-based linear models (DISTLM), on the basis of Bray-Curtis dissimilarity. For model selection we used the *Best* routine and the adjusted R^2 criterion applied to sequential tests of all possible combinations among variables. The faunal predictor variables included the population parameters life span, asymptotic length ($L\infty$), adult size (T_{ad}), growth performance index (phiL) and biomass; and the fatty acids were represented by cholesterol, cholestene, palmitic acid, tetradecanoic acid, pentadecanoic acid and a group of hormones (composed of androstanediol, estratrienone, prostadienoic acid and pregnene-trione). A permutation-based test was further carried out using Spearman rank correlations computed by the submodule BEST (Clarke and Gorley 2006), to obtain the significance *P*-value for the most parsimonious model given by *Best* selection. A distance-based redundancy analysis (dbRDA) with multiple partial correlations was then performed to visualize these relationships. All permutation-based tests were conducted under 9999 permutations. PERMANOVA, DISTLM, dbRDA and BEST were performed in PRIMER v6 with the PERMANOVA+ add on (Anderson et al., 2008).

Results

The population parameters for *L. acuta* life span, S_i, S_{ad}, $L\infty$, phiL, K, T_{anc}, Ts, Z and Rn, as well as mean biomass and density are shown in Table 1. Overall, despite its presence in relatively high densities throughout all months and estuaries, the population parameters for the species were significantly different between the urbanized and non-urbanized sites according to the PERMANOVA results (Table 2). The population patterns in the estuaries less affected by urbanization pressures were of lower life span (17 to 22 months), size at different life stages (0.027 to 1.005 mm), $L\infty$ (3.32 to 3.77 mm) and phiL (0.3 in average), along with higher growth rates (0.14 in average) and mortality rates (1.44 to 2.38). Oppositely, the patterns for the urbanized populations were of higher life span (25 to 26 months), size at different life stages (0.031 to 1.421), $L\infty$ (4.24 to 4.35) and phiL (0.34 in average), with lower growth (0.12 in average) and mortality (0.5 to 0.8) rates. The goodness of fit values (Rn) for all estuaries were in general high, ranging between 0.897 and 0.941.

Table 1. Population parameters for *L. acuta* in Urbanized and Non-urbanized estuaries. S_i = initial size of individuals; S_{ad} = size of adults (set by size of the smallest ovigerous females); $L\infty$ = asymptotic length (maximum size of individuals); phiL = growth performance index; K = growth coefficient; Ts = summer point; Z = mortality rate; Rn = goodness of fit values; B = biomass.

Condition	Site	Life span (mo)	$S7_i$ (mm)	$S7_{ad}$ (mm)	$L\infty$	phiL	K (mo)	T_s	Z	Rn	B (g)	Density (inds/m^2)
Non-urbanized	**Leste**	17	0.027	1.005	3.32	0.30	0.14	0.51	2.38	0.919	1.96	37.9
	Ratones	21	0.165	0.926	3.69	0.31	0.14	0.70	1.66	0.924	0.79	39.8
	Massiambu	22	0.084	0.517	3.77	0.30	0.14	0.58	1.44	0.897	5.49	183.3
Urbanized	**Itacorubi**	26	0.145	1.243	4.24	0.33	0.12	0.61	0.50	0.937	5.03	47.8
	Maruim	25	0.047	1.060	4.27	0.34	0.12	0.65	0.78	0.941	18.84	424.0
	Aririu	26	0.031	1.421	4.35	0.34	0.11	0.67	0.80	0.941	11.22	190.0

Table 2. PERMANOVA results for *L. acuta* population parameters at the different investigated Conditions (Urbanized x Non-urbanized estuaries). *p* values calculated through the Monte Carlo permutation test. Df = degrees of freedom; MS = mean square.

	df	MS	F	*p* (MC)
Condition	1	202.86	7.19	***0.02***
Estuaries	4	28.20		
Total	5			

The patterns of lower x higher mortality rates (Z) of *L. acuta* from urban to non-urban estuaries were additionally followed by a second common trend of more intense mortality rates in juveniles (0.5 to 1.66) than in adults (0.05 to 0.72), as shown in Fig. 2. The younger individuals (first equation) in general die faster than the older ones (second equation).

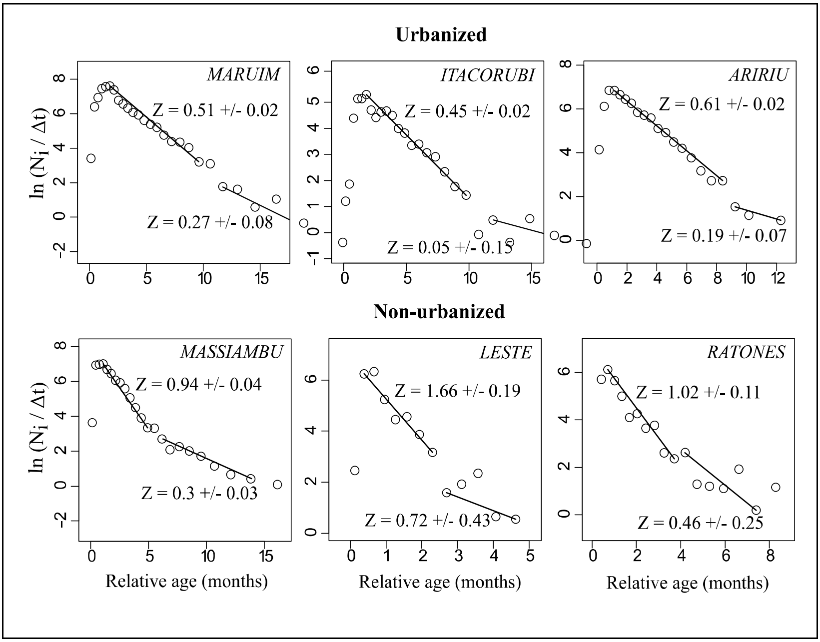

Fig. 2. Patterns of mortality of subsequent age classes in *L. acuta* from Urbanized and Non-urbanized estuaries. Z = mortality rate.

The dbRDA ordination between the faunal population parameters and the sediment fatty acids showed that 93.7% of the total variation of the data was explained in the DISTLM model, 88.5% for axis 1 and 5.2% for axis 2 (Fig. 3). The *L. acuta* parameters were more closely correlated to only 4 of the original 6 fatty acids, namely: palmitic acid, cholestene, cholesterol and the hormones group (Spearman correlation test; $p<0.05$). These compounds structured the samples mainly along the first axis (dbRDA1), with samples from urban sites grouped away from non-urban samples. In this estuarine gradient, all fatty acids were in general less related to the non-urbanized sites, mainly influenced by the decreasing presence of palmitic acid, as a possible sign of ambiguous natural and anthropogenic origins for the compound. However, the strongest correlation was the positive relationship between the hormones and the three urbanized estuaries along dbRDA2 axis. The mean values of the population parameters (phiL, L∞, life span, biomass and adult size) increased, as expected, from non-urbanized to urbanized estuaries.

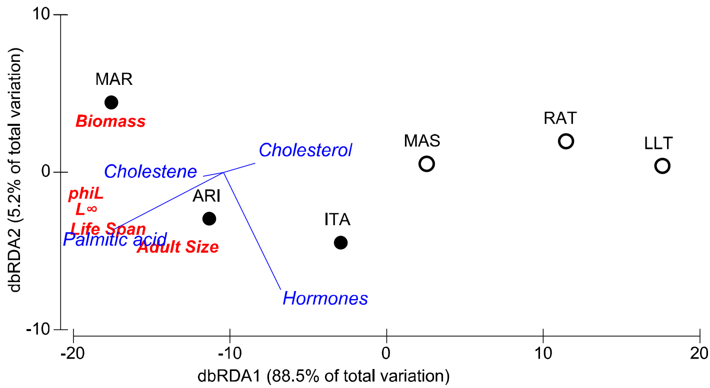

Fig.3. Distance-based Redundancy Analysis (dbRDA) on *L. acuta* population data for Urbanized and Non-urbanized estuaries. Vectors indicate the environmental variables that explained most of the variability in the dataset. Vector length corresponds to the relative size (or strength) of the effect, whereas direction represents the correlations to the two displayed axes.

Discussion

Despite relatively common and abundant in estuarine areas subjected to various sources and levels of impacts, responses of *L. acuta* population dynamics to the interaction between natural and urbanization (or pollution) pressures are seldom known. Our results show congruent opposite patters of population dynamics for the species in three urbanized and three non-urbanized estuaries. Differences in population parameters of *L. acuta* along the Southeastern Atlantic coast (Ieno et al., 2000; Omena and Amaral, 2000; Santos et al., 2002; Martin and Bastida, 2006) have already been found, suggesting that different environments are likely to impose shifts in the species' life history patterns.

The polychaete *L. acuta* is widely known as an opportunistic and/or resistant species that can proliferate in disturbed sites, and is even used in protocols for environmental quality assessment (Amiard-Triquet et al., 2013; Borja et al., 2000; Dauvin and Ruellet, 2007). However, in this study the populations from less affected estuaries displayed shorter life spans and smaller sizes along with higher growth and mortality rates, oppositely to the longer life spans, larger sizes with lower growth and mortality rates in the urbanized sites. These patterns disagree with the description of r-strategists, strongly referred as true opportunistics, as species of rapid growth and mortality, small body size and shorter life span (Pianka, 1970), more likely to occupy the urban instead of the non-urban sites.

The *L. acuta* ability to persist in urbanized estuaries has been previously linked to increases in biomass and body size as a possible physiological response to both organic enrichment and pollution, since larger organisms could be better buffered from changes in their physical environment (Weis et al., 2017). In addition, our results showed a positive correlation between increasing levels of four fatty acids with higher population biomass, adult size and life span in urban estuaries. Nevertheless, these fatty acids were represented by palmitic acid, cholesterol, cholestene and hormones, which can derive from both natural and anthropogenic sources, possibly linked to processes from plants and fungus decomposition to animal metabolism and artificial synthesis (Ahmad, 2017). For instance, the highly abundant palmitic acid is the major fatty acid found in several organisms such as phytoplankton, higher plants and composing the membrane of animal cells, likely to indicate either natural contributions and sewage discharges to the urban estuaries (Pinturier-Geiss et al., 2002).

Nevertheless, apart from its natural or artificial origin, the hormones were mostly related to the population responses within the three urban sites, and might be bioactive or disrupt metabolic pathways even in very low concentrations (Löffler et al., 2005). One of the hormones identified in this study, androstanediol, has already been found in the bivalve *Mytilus edulis*, with the potential to ultimately alter steroid hormone-dependent processes such as growth, development, and reproduction (Janer et al., 2005; Lafont and Mathieu, 2007). If discharged in domestic effluents, these hormones could change invertebrate population dynamics and ecosystem functioning as urban areas develop.

The consistency between the *L. acuta* population parameters biomass, adult size, life span, maximum length and performance of growth and these four fatty acids of along the urbanization gradient suggest that these fatty acids may not be considered unequivocal chemical proxies alone, since they might originate from both natural and human sources. Nevertheless, significant macrobenthic changes in virtue of other contaminants such as nutrients (P, N) and metals (Pb, Cr, Al, Cu, Cd) on the exact same estuaries have been previously found (Pagliosa et al., 2006; Weis et al., 2017), which sustain high background values of pollutant impacts linked to urbanization. The local distribution of some fecal sterols, much more conservative markers than biological or other physico-chemical variables, also support these assumptions (Mater et al., 2004). Another study in a subtropical Brazilian Bay using fatty acids as markers demonstrated that detritus pathways are more important than phytoplankton-derived routes in sustaining biomass and the development capacity of this system (Venturini et al., 2012)

The shifted population dynamics responses from urbanized to non-urbanized estuaries can also imply an essential difference between opportunism and tolerance. Despite indiscriminately used as synonyms, both strategies are in fact likely to reflect two distinct survival mechanisms: the first related to species life history (e.g. growth, mortality and reproduction mode) and the second more related to physiological mechanisms of tolerance to any xenobiotic. In their classical study on macrofaunal responses to enrichment impacts, Pearson and Rosenberg (1978) recognized a distinction between disturbance and enrichment opportunists. In this sense, *L. acuta* could benefit from human effluent inputs even behaving more like a typical k-strategist in the urbanized sites. Although the ability of this species to thrive in habitats with little anthropogenic pressure is not especially surprising, the life strategy in this case was more of k-selectors. Therefore, this polychaete may overlap changes in both population dynamic patterns (from k- to r-strategy) and physiological mechanisms of tolerance as simultaneous responses to thrive under stress.

The only common population pattern between the urban and non-urban sites emerged by taking a closer look into the general mortality rates in the six estuaries, which showed that in all populations the younger individuals died faster than the older ones. Despite the changes in population parameters when subjected to urbanization pressures, like in any other populations the polychaete die slower while aging (Sparre and Venema, 1998), and this congruent response could be interpreted as a sign of reliability of our data modelling.

We provided multiple lines of evidence indicating that *L. acuta* population dynamics varied according to the urbanization levels of estuaries, and were influenced by the distribution of sediment fatty acids from both natural and human origin. However, despite widely known as an opportunistic species, *L. acuta* population parameters varied contrarily to r- and k-strategists expectations, as a possible overlap in adaptive life strategies and plastic physiological mechanisms of tolerance. Higher biomass, size and life span within the urban estuaries can also represent population responses to local increasing inputs of hormones. We also emphasize the importance of using unequivocal chemical markers along with these fatty acids to establish more robust connections to the population dynamics directly driven by human waste contamination and ecotoxicological tests, in future surveys. Our results based on robust data interpretations also highlight that besides underpinning species life history patterns that maintain ecosystem outputs and functions, the parameters of population dynamics for this polychaete may directly indicate the environmental quality linked to anthropogenic use.

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CONCLUSÕES GERAIS

Este trabalho temo como objeto geral avalaiar as respostas aos efeitos da urbanização em diferentes níveis de complexidade biológica do poliqueta *Laeonereis acuta*. Para isto foram realizadas atividades de campo e laboratório em dez estuários da região Sul do Brasil.Nesse sentido, uma ampla variedades de técnicas e abordagens metodológicas foram empregadas. Além destas, foi desenvolvido um protocolo para a realização de teste do micronúcleo em invertebrados, obtendo células a partir de tecido regenerado.

As hipóteses de que *L. acuta* é uma espécie tolerante, e que consegue aproveitar eficientemente os recursos provenientes do enriquecimento promovido pela urbanização, agora se confirma através da análise dos parâmetros populacionais e da análise da capacidade de realização de trade-off realizado. Nesse sentido, esta espécie é capaz de desenvolver-se em abientes com muito pouco recurso, alocando suas reservas energéticas para a reprodução, enquanto que em locais mais ricos, o recurso é alocado para o crescimento somático e a longevidade.

O resultado do presente trabalho, visa contribuir com aumento do conhecimento sobre estuários, focando-os como ecossistemas dinâmicos de acoplagem entre o continente e o oceano. Também fonece nova abordagem para o estudo de danos moleculares, especialmente aqueles decorrentes da quebra e perda de material genético. A obtenção células mitóticas a partir da desagregação de tecidos regenerados visa contribuir de maneira simples, econômica e rápida nas respostas a contaminantes, especialmente metais pesados.

O uso do poliqueta *Laeonereis acuta* como bioindicador de qualidade ambiental, e as respostas em todos os níveis de organização biológica testados, representa um avanço no uso desta espécie como candidato a ser utilizado em programas de monitoramento e conservação de ambientes estuarinos. A capacidade de tolerar agentes xenobióticos utilizando com eficiência os recursos disponíveis am ambientes urbanizados, convertendo os produtos decorrentes do esgotamento doméstico requer mais estudos e a a possível indicação como agente para a renovação de ambientes degradados faz desta espécie um caditado importante na cadeia produtiva da aquicultura, especialmente no cultivo de camarão.

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